CHAPTER 4

Adipose Tissue and Adipocyte Differentiation: Molecular and Cellular Aspects and Tissue Engineering Applications

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Summary

Human adipose tissue represents an abundant, practical and appealing source of donor tissue for autologous cell replacement. Recent results have shown that stem cells within the stromal-vascular fraction of adipose tissue display a multilineage developmental potential. Adipose tissue-derived stem cells can be differentiated towards adipogenic, osteogenic, chondrogenic, myogenic and neurogenic lineages. To take full advantage of this new technology, it will be necessary to understand adipose tissue-specific signalling cascades and genes regulating adipose tissue-derived stem cell differentiation to various mesenchymal lineages. Adipocyte differentiation is an ordered multistep process requiring the sequential activation of several groups of transcription factors, including CCAAT/enhancer-binding protein (C/EBP) gene family and peroxisome proliferator-activated receptor-γ (PPAR-γ). Hormones and growth factors that affect adipocyte differentiation, such as insulin and insulin-like growth factor, transfer external growth and differentiation signals to differentiating adipocytes. In addition, extracellular matrix proteins are also important in regulating the differentiation process. Several preadipocyte and stem cell culture models have been developed to improve the quality of tissue-engineered fat by culture-expanded adipocytes. Recent advances in bioengineering and cell biology of adipose tissue have led to new therapeutic potentials for regenerative medicine.

KEYWORDS: Adipocyte, tissue engineering, adipose tissue, regeneration

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Introduction

Fat tissue engineering offers great potential in repealing limitations realized with classical approaches in reconstructive surgery. The clinical applications for tissue engineered fat are vast and variable, including reconstructive, cosmetic and corrective indications. Adipose tissue represents an ideal source of autologous cells for tissue engineering strategies because of its unique accessibility and expendability. Adipose tissue is particularly easily obtained in large amounts using the techniques of liposuction. Fat tissue contains numerous different cell types that may be advantageous to tissue engineering applications and regenerative medicine. In recent years, adipose tissue-derived stem cells have been cultured and differentiated into several lineages, such as fat, bone, cartilage, muscle and neuronal cells. Promising results indicate that these stem cells have therapeutic potential and utility for future tissue engineering applications and cell-based therapies.

Adipocyte differentiation is characterized by sequential changes in the expression of specific genes that determine the specific adipocyte phenotype of the cells. This is reflected by the appearance of various early, intermediate and late mRNA/protein markers and triglyceride accumulation. The regulation of adipocyte genes occurs primarily at the transcriptional level. Several transcription factors that play a central role in the control of adipogenesis have been identified. Among these are CCAAT/enhancer binding protein (C/EBP) gene family and peroxisome proliferator-activated receptor γ (PPAR-γ) that coordinate the expression of genes that create and maintain the adipocyte phenotype. During adipocyte differentiation, remarkable changes occur also in cell morphology, cytoskeletal components and the level and type of extracellular matrix (ECM) components secreted. A thorough understanding of adipose tissue on a cellular and molecular level and mimicking the events of natural development is essential for regeneration of additional and self-regenerating adipose tissue for reconstructive or cosmetic purposes.

Adipose tissue structure and function

Adipose tissue is the most prevalent tissue in the human body. It is commonly found in subcutaneous loose connective tissue, and it also surrounds internal organs. Mature adipocytes constitute the majority of cells in adipose tissue. Besides mature adipocytes, fat tissue contains several other cell types, including stromal-vascular cells (SVC) such as fibroblasts, smooth muscle cells, pericytes, endothelial cells, and adipogenic progenitor cells

or preadipocytes.[76] Recent research shows that adipose tissue plays a more dynamic role than previously recognized in physiological processes of the whole body.

Adipose tissue is divided into two subtypes, white and brown fat. White fat is widely distributed and it represents the primary site of fat metabolism and storage, whereas brown fat is relatively scarce and its main role is to provide body heat, which is essential for newborn babies. White adipose tissue is the major energy reserve and its primary function is to store triacylglycerol (TG) in periods of energy excess and to release energy in the form of free fatty acids during energy deprivation.[51, 141] Fat tissue also plays an important role in numerous processes through its secretory products and endocrine functions. Adipocytes secrete various factors known to play a role in immunological responses, vascular diseases and appetite regulation. Leptine is a peptide hormone primarily made and secreted by mature adipocytes, and it has various biological activities, including effects on appetite, food intake and body weight regulation, fertility, reproduction and hematopoiesis.[39,83] Adipose tissue is an important site for oestrogen biosynthesis and steroid hormone storage.[28,87,114] In addition, adipose tissue secretes a variety of peptides, cytokines and complement factors, which act in an autocrine and paracrine manner to regulate adipocyte metabolism and growth, as well as endocrine signals to regulate energy homeostasis.[51,83]

Although adipose tissue is vitally important to various normal processes of the human body, it has also many implications for human disease states. Obesity is a common health problem in industrialized countries and is considered a major risk factor for noninsulin-dependent diabetes mellitus,[105] cardiovascular diseases and hypertension.[157] Obesity has also been associated to other pathological disorders, including some types of cancer, such as breast, ovarian, renal and colon cancer.[13,31,74,162]

The adipogenic lineage and in vitro models of adipocyte differentiation

Several studies on multipotent clonal cell lines suggest that the adipocyte lineage is derived from an embryonic stem cell precursor with the capacity to differentiate into the mesodermal cell types of adipocytes, chondrocytes, osteoblasts, and myocytes.[51,89] Adipoblasts, which are the earliest unipotential cells believed to belong to the adipogenic lineage and are thought to be derived from mesodermal stem cells, can commit to the adipogenic lineage and become preadipocytes. In a suitable micromilieu, preadipocytes can differentiate into mature, lipid-synthesizing and lipid-storing adipocytes.[77] The growth of white adipose tissue is a result of both increased adipocyte size as well as an increase in adipocyte number. The potential to

produce new fat cells from fat cell precursors continues throughout the lifespan.[102,130,131] While molecular pathways that are important in later stages of adipocyte differentiation have been identified, the early molecular events that promote commitment of mesenchymal precursor cells to the adipogenic lineage are not well established.[47]

Almost all work on adipogenesis has used either predetermined clonal cell lines or primary cultures of adipose tissue-derived stromal-vascular precursor cells, which have been successfully cultured from a number of species including humans.[45,48,57,116] Cultures derived from primary tissues contain cells at different stages of development and can not be as scientifically controlled as preadipose cell lines, but they probably reflect more accurately the normal condition *in vivo*.[15] In addition, primary cells can be isolated from various species and from different fat depots, as well as from animals of different physiological states and ages, allowing comparisons between cells of different origins.

During growth, cells of preadipose cell lines as well as primary cultures of adipose-derived precursor cells morphologically resemble fibroblasts. At confluence, induction of differentiation by appropriate treatment leads to conversion of the cells to a spherical shape accumulating lipid droplets and acquiring the morphological and biochemical characteristics of the mature white adipocyte (Fig. 1). Various differentiation protocols have been developed for preadipose cell lines and primary cultures of adipose-derived precursor cells, and the responsiveness of preadipocytes from various sources to inducing agents may vary considerably.[51] In the presence of fetal calf serum, spontaneous differentiation of preadipocytes into fat cell clusters occurs to some degree. The amount of lipid synthesis can be controlled, in a dose-dependent manner, by varying the amount of serum in growth media. Differentiation can be enhanced by the inducing agents such as dexamethasone, which is used to stimulate the glucocorticoid receptor pathway, and 3-isobutyl-1-methylxantine (IBMX) (or 1-methyl-3-isobutylxanthine, MIX), which is used to stimulate the cAMP-dependent protein kinase pathway. High concentrations of insulin have also been used in combination with these inducing agents.[137,140] The involvement of insulin/insulin-like growth factor 1 (IGF-1), glucocorticoid and cAMP signalling pathways in the adipocyte differentiation process has been confirmed.[51,117]

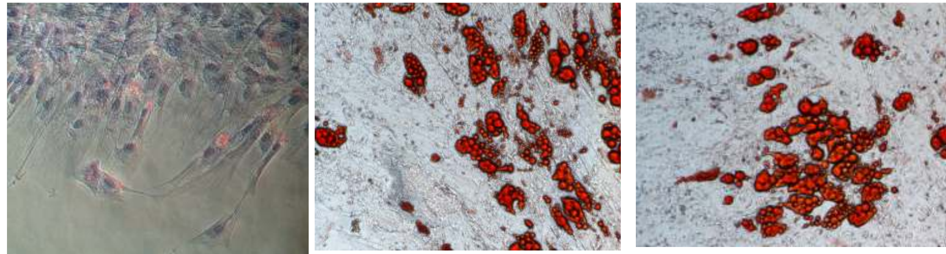

Fig. 1. Human stem cells were harvested from subcutaneous adipose tissue and cultured in adipogenic conditions for one (A), two (B) and three (C) weeks. The differentiated cells accumulated lipid droplets as demonstrated by Oil Red O staining (Sarkanen *et al*., unpublished data)

Program of adipocyte differentiation

Committed preadipocytes have to withdraw from the cell cycle before adipose conversion. Upon reaching confluence, proliferative preadipocytes become growth-arrested by contact inhibition. Those cells re-enter the cell cycle after hormonal induction, stop proliferating again and undergo terminal adipocyte differentiation. Re-entry into the cell cycle of growth-arrested preadipocytes is known as the clonal expansion phase.[35] Adipocyte differentiation is characterized by sequential changes in the expression of specific genes that determine the specific adipocyte phenotype (Fig. 2). These changes in gene expression occur primarily at the transcriptional level and are reflected by the appearance of early, intermediate and late mRNA and protein markers and TG accumulation. Changes in gene expression during the different stages of adipocyte differentiation have been characterized mainly through the use of preadipose cell lines.[99]

Transcriptional regulation of adipocyte differentiation

Several transcription factors that regulate adipocyte differentiation have been identified. Two transcription factors, CCAAT/enhancer binding protein α (C/EBP-α) and peroxisome proliferator-activated receptor γ (PPAR-γ) have been shown to activate adipocyte-specific genes and are involved in the growth arrest that is required for adipocyte differentiation. They appear to act cooperatively in adipocyte differentiation by activating the expression of one another and regulating the expressions of other adipocyte specific genes.[100] PPAR-γ is the most specific to adipogenic differentiation and is induced before transcriptional activation of most adipocyte genes.[51] Activated PPAR-γ induces exit from the cell cycle and triggers the expression of adipocyte-specific genes, resulting in increased delivery of energy to the cells.[36] Transcription factors that belong to the C/EBP family of DNA binding proteins also play an

important role in adipocyte differentiation. C/EBP-α is expressed slightly before the transcription of most adipocyte specific genes is initiated, and it has been shown to be required for the adipogenic induction.[96,140] It has been demonstrated that C/EBP family members C/EBP-β and C/EBP-δ are involved in adipogenic induction at an earlier stage than PPARγ, and that the promoter region of the PPARγ gene has binding sites for C/EBP.[100,181] The role of adipocyte determination- and differentiation-dependent factor-1/sterol regulatory element-binding protein-1 (ADD-1/SREBP-1) in adipocyte differentiation has also been indicated. ADD-1/SREBP-1 is induced very early during adipocyte differentiation and may also participate in adipocyte gene expression. ADD-1/SREBP-1 clearly stimulates the expression of many of the genes necessary for lipogenesis *in vivo*.[34,82] C/EBP-β, C/EBP-δ and ADD-1/SREBP-1 induce the expression and/or activity of PPARγ, the pivotal coordinator of the adipocyte differentiation process.[36,145] A steroid receptor coactivator-3 (SRC-3) has a strong impact on the white adipocyte formation.[97] The differentiation of the white adipocytes is completely inhibited in cultured mouse embryonic fibroblasts in the absence of SRC-3. At the molecular level, SRC-3 acts synergistically with the transcription factor C/EBP to control the gene expression of PPARγ2.

Lipoprotein lipase (LPL) catalyzes the hydrolysis of TG molecules while it is associated with capillary endothelial surfaces, and it is abundant in adipose tissue. The expression of LPL mRNA has often been considered as an early sign of adipocyte differentiation.[99] LPL is secreted by mature adipocytes and is important in controlling lipid accumulation.[44] Preadipocyte factor-1 (pref-1) has been shown to participate in maintaining preadipose phenotype. A decrease in pref-1 expression is observed during adipocyte differentiation.[152] Recent findings have demonstrated the inhibitory effect of pref-1 on adipogenesis *in vivo*.[173]

During the terminal phase of differentiation, adipocytes in culture markedly increase *de novo* lipogenesis and become sensitive to insulin. The activity levels of proteins and mRNAs for enzymes involved in TG metabolism including adenosine triphosphate (ATP) citrate lyase, malic enzyme, glycerol-3-phosphate dehydrogenase and fatty acid synthase increase.[51,124,156] Adipocytes also synthesize other adipose tissue-specific products that are not directly related to lipid metabolism. These include aP2, an adipocyte-specific fatty acid-binding protein that has been considered as an intermediate marker of adipocyte differentiation.[11,77,156] aP2 is the predominant fatty acid-binding protein found in adipose tissue, and it has an important role in the intracellular metabolism and transport of fatty acids.

The expression of aP2 is confined almost exclusively to adipose tissue and adipogenic cell lines and is highly regulated during adipocyte differentiation.[22,135] In addition, adipocytes produce various secreted factors including adipsin, angiotensinogen II and leptin that are regarded as late markers of adipocyte differentiation.[51,73,99] Acyl-coenzyme A (CoA)-binding protein (ACBP) is also considered as a late marker of adipogenesis and it is significantly induced during adipocyte differentiation.[55,77] ACBP regulates the availability of acyl-CoA esters for various metabolic and regulatory purposes and it has been shown to play a role during adipocyte differentiation.[101] PPAR-γ and C/EBP-α are involved in the coordinated activation of several of these genes, including aP2 and leptin.[51,67,157]

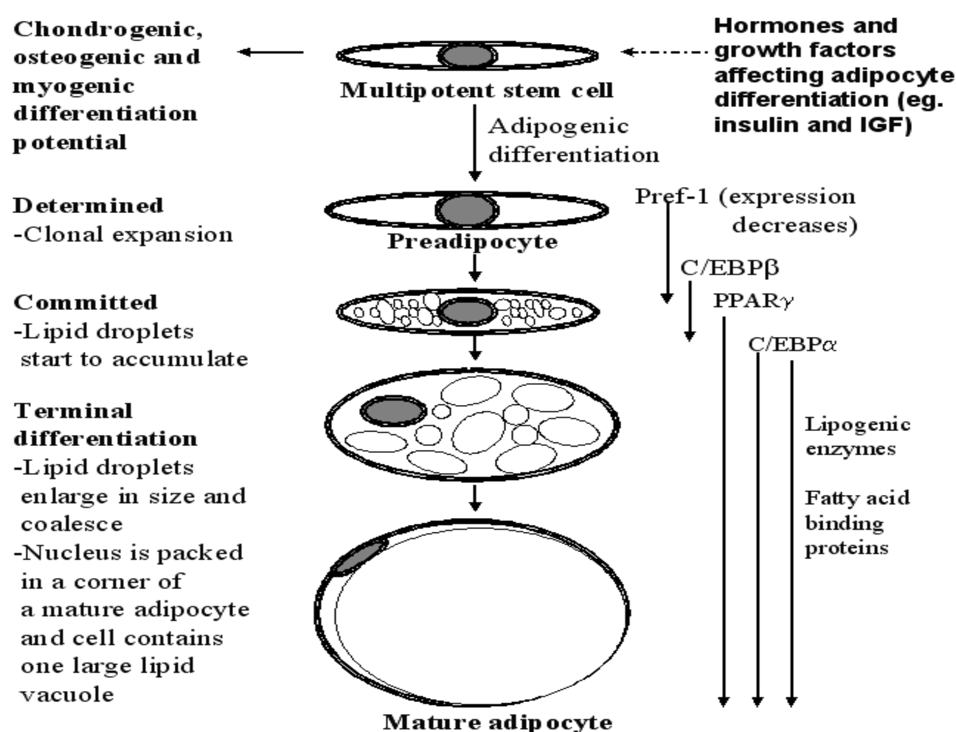

Fig. 2. An overview of the stages in adipocyte differentiation. Multipotent stem cell, with the capacity to differentiate along mesenchymal lineages of myoblast, chondroblast, osteoblast and adipocyte, gives rise to a preadipocyte. When exposed to appropriate environmental and gene expression conditions, these cells undergo clonal expansion and subsequent terminal differentiation; cells enlarge in size while accumulating lipid vacuoles that coalesce and eventually fill the cells. The molecular events accompanying this process are indicated on the right, with their approximate duration reflected by arrows. Abbreviations: IGF, insulin like growth factor; pref-1, preadipocyte factor-1; C/EBP, CCAAT/enhancer binding protein; PPARγ, peroxisome proliferator-activated receptor-γ.[113] (Reproduced with kind permission from the Editor-in-Chief of the Journal of Craniofacial Surgery. Original publication[113] J Craniofac Surg. 2007;18(2):325-335.).

Hormones and signal transduction pathways regulating adipogenesis

Hormones and growth factors with a role in adipocyte differentiation act via specific receptors, which mediate external growth and differentiation signals through a cascade of intracellular events. IGF-1 has been shown to be an essential regulator of fat cell formation, and the requirement of IGF-1 and insulin in adipocyte differentiation has been clearly demonstrated.[43,56,154,160] The effect of insulin on differentiation has been shown to occur through cross-activation of the IGF-1 receptor. IGF-1 and insulin activate several distinct downstream signal transduction pathways, which could mediate the adipogenic effects of these hormones.[51,140] However, stromal cells from different origin may respond to insulin stimulation differentially. For example, bone marrow-derived stem cells with demonstrated capacity to differentiate to adipocytes do not require insulin for adipogenic differentiation.[92]

Glucocorticoids have been used for many years to induce optimal differentiation of cultured preadipocyte cell lines and primary preadipocytes. Dexamethasone is believed to operate through activation of the glucocorticoid receptor, which is a nuclear hormone receptor in the same superfamily as PPAR-γ.[140] Dexamethasone has been shown to induce C/EBP-δ, which may account for some of its adipogenic activity,[179] and to reduce the expression of pref-1, a negative regulator of adipogenesis.[153] Isobutylmethylxantine (IBMX or MIX) has been shown to increase the expression of C/EBP-β, which is required for subsequent PPAR-γ expression and adipocyte differentiation.[16] Several studies have indicated that IBMX may function through increasing the accumulation of cAMP, which acts through cAMP response element-binding protein (CREB) and promotes the differentiation by inducing C/EBP-β. CREB has been implicated as a transcriptional activator in adipocyte differentiation program.[112,137]

Adipogenesis in rats has been demonstrated to be site-specifically controlled by the ovarian status, since ovary-removal induced mainly abdominal obesity,[91] and interestingly, oestrogen receptor amount varies between anatomical origins of isolated fat cells.[3,125] Oestrogen has been demonstrated to enhance human preadipocyte replication *in vitro*,[23] but oestrogen together with progesterone have no effect on adipocyte differentiation.[56] However, progesterone alone was found to stimulate adipogenesis in 3T3-L1 fibroblasts.[139] Growth hormone, retinoic acid, vitamin D and various prostaglandins are among a wide variety of other hormones that may affect adipogenesis.[46,80,88,136,143,150,171] Table 1 illustrates hormones and differentiation factors that are reported to affect adipocyte differentiation.

Adipose tissue extracellular matrix and its alterations in adipocyte differentiation

ECM provides structural support and tensile strength, attachment sites for cell surface receptors, and it is a source of signaling factors that modulate a variety of host processes such as angiogenesis, cell migration, proliferation and differentiation as well as immune responsiveness. ECM of adipose tissue interconnects adipocytes and leads to the formation of fat cell clusters *in vitro* and fat lobules *in vivo*. Two different parts of the ECM can be distinguished; the basement membrane surrounding individual adipocytes and the reticular fibre network. Fat lobules are highly vascularized and consequently endothelial cells and their ECM are also present.[141] During adipocyte differentiation, dramatic changes occur in cell morphology, cytoskeletal components and the level and type of ECM components secreted. One of the earliest changes seen in adipocyte differentiation is the deposition of collagen at the cell-ECM border and biogenesis of the basement membrane.[109] Several *in vitro* studies have shown that ECM molecules play an important role in regulating adipocyte differentiation. Alterations in the composition of ECM during adipogenesis have been shown to induce morphological changes and cytoskeletal reorganization of adipose cells leading to changes in adipocyte differentiation.[42,156] Modulation of ECM components could change cell adhesion properties and permit remodelling of cell components, leading to cellular reorganization and the expression of adipocyte genes.[51]

Table 1. Hormones and differentiation factors influencing adipocyte differentiation.

Agent	Effect	Comments	References
Insulin	+	Accelerates lipid accumulation	43, 57, 106, 154, 160
IGF-1	+	Stimulates adipocyte differentiation	132, 154, 170
Glucocorticoids	+	Stimulate adipocyte differentiation	49, 57, 106, 160, 179
Growth hormone	+/-	Induces adipogenesis in preadipose cell lines, inhibits adipogenesis in primary cultures	46, 60, 170, 171
Retinoic acid	+/-	Concentration dependent	143, 144
Thyroid hormone	+/no effect	Inducing effect on adipogenesis restricted to a preadipose cell line	57, 146, 148, 160, 175
Prostaglandins	+/-	Varied effects depending on model system	103, 110, 136, 167
EGF, TGF-α	-	Inhibit adipocyte differentiation	58, 98, 146, 167
TGF-β	-	Potent inhibitor of adipogenesis	127, 149, 155, 167
aFGF, bFGF	+/-	Conflicting results	58, 79, 146, 148, 167
IL-1, interferon-γ, TNF-α	-	Inhibit adipocyte differentiation	50, 119, 126
PDGF	+/-	Conflicting results	58, 61, 146
cAMP	+	Induces adipocyte differentiation	137, 175, 180
Vitamin D	+/-	Conflicting results	10, 80, 88, 169
Oestrogen, progesterone	+/no effect		56, 139

Abbreviations: IGF-1, insulin-like growth factor 1; EGF, epidermal growth factor; TGF, transforming growth factor; FGF, fibroblast growth factor; IL-1, interleukin-1; TNF-α, tumor necrosis factor-α; PDGF, platelet-derived growth factor; cAMP, cyclic adenosine monophosphate.

Studies on preadipocyte cell lines have shown that during adipocyte differentiation as preadipocytes lose their fibroblastic characteristics, the expression levels of collagen type I and type III decrease,[174] while the levels of type IV collagen, laminin, entactin and glycosaminoglycans increase during the differentiation process.[4,90,120] The $\alpha2$ chain of type VI collagen increase at confluence in preadipocytes and then gradually decrease.[26] The amount of pericellular fibronectin as well as cellular synthesis of fibronectin has been shown to decrease during the differentiation of preadipocytes.[2] A significant increase in the amount of type I-VI collagens, laminin and fibronectin has been demonstrated during adipocyte differentiation of a stromal-vascular preadipocyte cell line derived from bovine intramuscular adipose tissue. Indirect immunofluorescence staining indicated that collagen type IV progressed to a fibrillar network on the surface of adipocytes, and collagen type V and VI also formed a great number of fibers during the adipogenic process.[107] ECM has also been shown to have functional importance in adipocyte differentiation. Inhibition of collagen synthesis prevents adipocyte differentiation, demonstrating that active synthesis of collagen is also required for adipocyte differentiation.[71] Fibronectin has been shown to both inhibit and stimulate adipocyte differentiation.[42,156]

Fat tissue engineering

A large proportion of the plastic and reconstructive surgical procedures performed are to repair soft tissue defects resulting from traumatic injury, tumor resection, congenital defects or ageing process. Transplantation of autologous fat tissue grafts has been the classical method for soft tissue reconstruction and plastic surgery. Despite the efforts toward improving this procedure, problems such as progressive absorption of fat grafts with time have been observed.[77] The reduction in adipose volume is thought to be partly related to insufficient vascularization of grafted fat tissue.[111] Fat tissue is highly vascularized with extensive capillary networks surrounding each adipocyte, and fat tissue itself has angiogenic properties.[25,151] Innervation is also an important feature for adipose tissue. There is strong evidence for the role of the autonomic nervous system in modulating the fundamental properties of adipose tissue function and biology at the cellular and molecular level. This is reflected in the modulation of lipolysis/lipogenesis, local insulin sensitivity of glucose and fatty acid uptake, and the modulation of fat cell number.[9,40,134,138] The potential development of tissue-engineered soft tissue represents a promising and innovative solution for many clinical challenges, especially in plastic and reconstructive surgery.

Potential applications of tissue-engineered fat include reconstructive, cosmetic and corrective indications. Congenital deformities, complex traumatic wounds involving soft tissue defects and post-cancer surgery are reconstructive challenges potentially benefiting from soft tissue engineering strategies. Cosmetic applications include augmentation procedures for lips and chin, and rejuvenation procedures to fill out wrinkles of the aging skin. Correction uses of engineered fat might include the treatment of urinary incontinence or vocal cord insufficiency, in which a stable, long-lasting "bulking agent" is needed.[77,121]

There are two possible research strategies of tissue engineering to induce *de novo* adipogenesis. One method is to use cells that proliferate and differentiate to form adipose tissue. Cells isolated from a patient's own tissue are grown in culture and seeded onto a biocompatible scaffold with[66,84,85,158,166,172] or without bioactive molecules such as growth factors. Engineered implant is then brought into a body site where the formation of adipose tissue is expected.[77] For example, adipose tissue has been formed in the subcutis of rats by seeding autologous preadipocytes on poly(lactic-co-glycolic acid) scaffolds.[122] Alternatively, adipose tissue formation could be induced *in vivo* from precursor or stem cells originally existing in the body. Site-specific delivery of potent bioactive factors that influence the growth and development of *in vivo* progenitor or stem cells in a specific manner could provide a suitable method for *de novo* formation of adipose tissue.[77] It has been reported that *de nov*o adipogenesis in the subcutis of mice could be achieved by injection of a mixture of basement membrane extract "Matrigel" and basic fibroblast growth factor (bFGF) incorporated into biodegradable microspheres.[161]

Biomaterials used for adipose tissue engineering may either be fibrous scaffolds or injectable materials, such as hydrogels,[65] containing cells and adipogenic or angiogenic factors.[121] They can be either of natural (Fig. 3) or synthetic origin. Reports on naturally-derived biomaterials for fat tissue engineering include hyaluronan-gels,[65] sponges,[53,62,63] or nonwoven carriers,[62] Matrigel,[81,84,158,172] collagen type I matrix,[166] collagen sponge,[66,85] gelatin sponge,[68,69] decellularized ECM of human placenta,[41] collagen:chitosan blend,[178] fibrin,[18,19,163] and alginate gels.[52] Synthetic biomaterials that were tested for fat tissue engineering include polyglycolide (PGA) scaffolds,[37,38] poly(lactide-co-glycolide) (PLGA) scaffolds,[30,122,123] PLGA spheres,[20] polyesteramide-derived nonwovens,[64] poly(ethylene glycol)-based hydrogel,[159] perfluoroelastomer,[21] or nonbiodegradable fibrous polyethylene terephthalate scaffolds.[75] More recently, scaffolds that are based on utilising thinner fibres became available. They are thought to mimic the nanoscale of fibres found in natural ECM and are being tested for engineering of various tissue types.[5,6] Some authors,[168] however, used

a strategy that involves concomitant induction of adipogenic differentiation and ascorbic acid stimulation of stromal cells to produce and organize their own ECM sheets that are then assembled into thicker reconstructed adipose tissues.

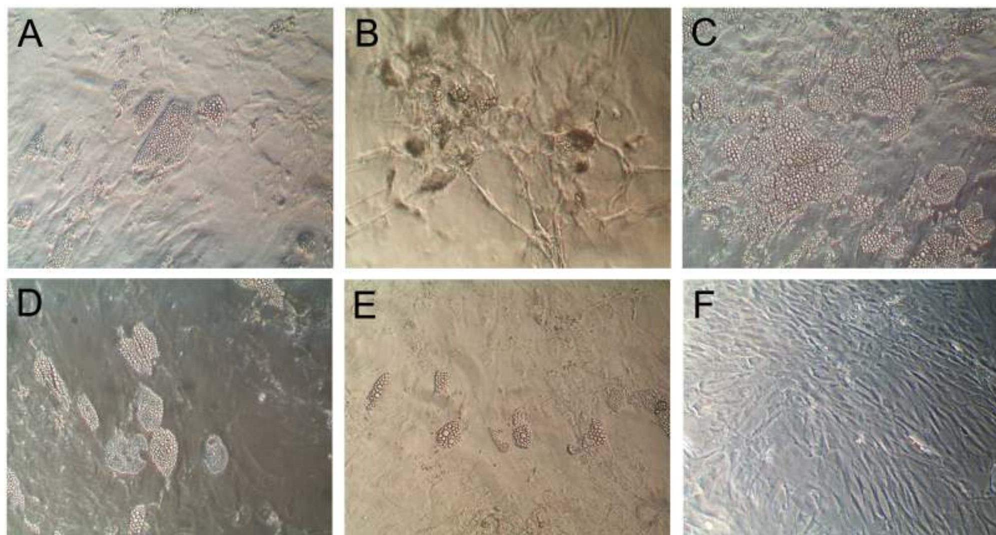

Fig. 3. Adipocyte differentiation of human adipose tissue-derived stem cells on extracellular matrix substrata. Cells were cultured in adipogenic conditions for ca. two weeks on substrata of Matrigel applied in the adipocyte medium (A) and used as a thin gel coating (B), Human placental ECM applied in the adipocyte medium (C) and used as a thin coating (D), untreated tissue-culture plastic (E), or undifferentiated culture on tissue-culture plastic (F) (Magnification 100×) (Niemelä *et al.*, unpublished data).

Applications of fat tissue-derived cells for cell-based therapy

Adipose tissue provides a uniquely abundant and accessible source of autologous cells for applications in tissue engineering and regenerative medicine. Adipose tissue can be harvested in large amounts with minimal morbidity. It contains several cell types, including mature adipocytes and stromal-vascular cells (SVC) such as fibroblasts, smooth muscle cells, pericytes, endothelial cells and preadipocytes that may be advantageous to soft tissue regeneration.[76] Preadipocytes are fibroblast-like cells that can be isolated from adult white adipose tissue of various species, including humans, and are able to proliferate and differentiate into mature, lipid-synthesizing and lipid-storing cells both *in vitro* and *in vivo*.[129,164,165]

There are documented differences in the growth and differentiation of adipogenic progenitor cells derived from different adipose tissue sites.[1,48,94] Studies on the responsiveness of SV cultures derived from pig adipose tissue to adipogenic agents have

demonstrated several genotype- and age-dependent characteristics.[59] The differentiation capacity of primary preadipocyte cultures has been shown to be donor-dependent and decrease with age.[27,86] Human adipose tissue-derived stromal cells isolated from multiple donors have been shown to display varying degrees of differentiation in response to an optimal adipogenic stimulus *in vitro*.[147] It has been proposed that cells from different donors may be arrested at distinct stages of adipocyte development and therefore require a different subset of signals to undergo adipocyte differentiation.[32,51] Rat primary subcutaneous preadipocytes in culture display a higher capacity to differentiate than epididymal preadipocytes.[48] It was shown that human preadipocytes from different sites of the same subject respond differently to a specific adipogenic stimulus on molecular level. As assessed by lipid accumulation, lipogenic enzyme activity and mRNA levels, preadipocytes from subcutaneous sites were much more responsive to specific adipogenic compounds when compared to preadipocytes derived from omental fat of the same individuals.[1] Differences in the expression of mRNAs encoding a number of proteins involved in the control of adipocyte metabolism, including leptin and glycogen synthase, have been demonstrated between human subcutaneous and omental adipose tissue.[94] Further work could possibly reveal optimal harvest sites for tissue engineering applications of adipose-derived precursor cells.

Adipose tissue as a source of multipotent stem cells

Recent evidence supports the existence of multipotent adult progenitor cells in various depots of the body, such as skeletal muscle, bone marrow, synovial tissue, and periostium.[12,72,95,108,115] It has been recently acknowledged that also human adipose tissue contains a population of multipotent stem cells that can be isolated in significant numbers from adipose tissue obtained by liposuction or biopsy, and expanded on culture. These stem cells can be differentiated successfully into various mesenchymal cell lines *in vitro* when exposed to specific growth conditions, and they represent a promising option for tissue engineering applications. Recent results have shown that human adipose tissue-derived stem cells can be differentiated *in vitro* towards adipogenic,[182,183] osteogenic,[54,118] chondrogenic,[8,33,70,118,176] myogenic,[104,183] cardiomyogenic,[133] and neurogenic lineage.[7,142,183] Interestingly, in many of these *in vitro* differentiation conditions nearly similar composition of differentiating agents were used, but the concentrations were different.

The cell surface phenotype of human adipose tissue-derived stem cells is quite similar to bone marrow-derived mesenchymal stem cells (MSC). The stem cell population derived from adipose tissue has been shown to express multiple stem cell-related surface marker

antigens similar to those observed on MSCs. However, adipose-derived stem cells also exhibit unique characteristics distinct from those seen in MSCs, including differences in gene expression.[29,183] Recently, undifferentiated human adipose-derived stromal cells were characterized on a transcriptional level by evaluating genes relating to angiogenesis and the ECM. The most highly transcribed genes related to functional groupings such as cell adhesion, matrix proteins, growth factors and receptors, and proteases, and the transcription in adipose-derived stromal cells had many similarities to the profile of MSCs.[78] The similarities between the phenotypes of human adipose tissue- and bone marrow-derived stem cells could have broad implications for human tissue engineering.

In addition to soft tissue reconstruction, adipose tissue-derived stem cells have a potential in repair of cartilage and bone. Under osteogenic conditions, adipose-derived stem cells are observed to express genes and proteins associated with an osteoblast phenotype, including alkaline phosphatise, osteopontin, and osteocalcin.[183] Osteogenic differentiation is characterized by acquisition of cuboidal osteoblastic morphology and deposition of a hydroxyapatite-mineralized ECM. Using appropriate supportive scaffold, human fat-derived stem cells can form bone in immunodeficient rodent ectopic bone models.[93] The chondrogenic differentiation is characterized by decrease of type I collagen and increase of type II, VI and IX collagen and aggrecan expression.[183] Adipose tissue-derived stem cells seeded onto alginate discs and implanted into immunodeficient mice exhibit prolonged synthesis of cartilage matrix molecules.[33]

In vitro differentiation along the neuronal lineages has also been demonstrated for adipose tissue-derived stem cells.[7,142,183] Neuronal induction of adipose-derived stem cells results in transition of cells to a neuronal morphology and expression of early markers of neuronal lineage. In addition to these findings, fat tissue-derived stem cells have been suggested to differentiate into myoblasts,[104] cardiomyocytes,[133] hematopoietic cells,[24] and macrophages.[17] Culture of adipose-derived stem cells in myogenic conditions has been shown to result in a time-dependent pattern of expression of muscle-related genes.[104,183] After three weeks differentiation into cardiomyocytes, the cells began to beat spontaneously and differentiated cells maintained their phenotype up to two months.[133] A recent study has suggested that adipose tissue contains a population of cells with haematopoietic stem cell activity, i.e. a population of cells capable of rescuing lethally irradiated animals.[24] Differentiation towards macrophage lineage was achieved when mice SVC were injected into the peritoneal cavity of nude mice, where these cells acquired phagocytic activity and started to express gene markers similar to macrophages.[17] Furthermore, cultured adipose-derived

stem cells can also be induced to differentiate into endothelial cells in certain conditions.[128] Interestingly, they secrete a number of angiogenesis-related cytokines, such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF), which could be suitable for regenerative cell therapy for ischemic diseases. However, there are conflicting data addressing the question if the endothelial and mesenchymal differentiation capacity within adipose tissue reside within the same cells. Wosnitza *et al*. (2007) have shown that both preadipocytes and endothelial cells share a common progenitor cell type demonstrating endothelial and adipogenic maturation potential. Furthermore their result reveals that even some mature cells of mesenchymal origin have a remarkable potency to perform transdifferentiation between endothelium and adipose tissue.[177] Boquest *et al*. (2006), have, however, shown that despite limited upregulation of endothelium related marker surface proteins CD31 and CD144 expression after endothelial differentiation stimulation, adipose tissue derived stem cells have a limited commitment to the endothelial lineage *in vitro*.[14]

Conclusions

Resent advances in bioengineering and cell biology of fat tissue have led to innovative and new therapeutic potentials for regenerative medicine. Autologous human adipose tissue-derived stem cells could have clinical applicability for cell-based therapies and tissue engineering purposes. Promising results suggest that adipose tissue will be a useful tool in biotechnology.

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