Fundamentals and Principles of Biomolecules in Adipose Stem Cell Engineering

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Contents

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10.1 Introduction

Human adipose tissue is comprised of three main fat deposits – visceral white fat, subcutaneous white fat, and brown fat – each with its own unique properties. In particular, white adipose tissue is associated with energy storage and hormone production, while brown adipose tissue is mainly responsible for heat production through energy expenditure (thermogenesis) [1]. Although many informative studies have been performed on cultured adipocytes, there are still some aspects of adipocyte function that require further investigation. For instance, the regulation of adipose tissue metabolism is controlled by activation of the autonomic nervous system, delivery of a complex mixture of substrates and hormones to adipose tissue, feedback from autocrine and paracrine effectors secreted by adipocytes, and the vascularity of the adipocytes [2]. Humans are born with a specific numeric amount of adipocytes that multiply and develop until puberty, subsequently remaining constant thereafter. Irrespective of exercise and/or strict dietary modification, humans cannot reduce the number of fat cells. Nonsurgical treatment such as aerobic exercise and balanced diet will eventually decrease adipose cell mass; however, the actual number of those cells will remain constant [3]. Adipose tissue contains adipose-derived stem cells, which possess the ability to differentiate into multiple cellular lineages, a property that represents the key to regenerative medicine. By definition, stem cells are characterized by their ability to undergo multilineage differentiation and form terminally differentiated cells. Guilak et al. assessed this potential by culturing and ring cloning to select cells derived from one progenitor cell. Forty-five clones were

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expanded through four passages and then induced for adipogenesis, osteogenesis, chondrogenesis, and neurogenesis using lineage-specific differentiation media. The authors found that 81% of adipose stem cell (ASC) clones differentiated into at least one of the lineages [4]. An ideal stem cell, one that can potentially be used in regenerative medicine, should have the following characteristics: (a) found in large quantities, (b) easily collected or harvested, (c) is differentiated into multiple cell lineage pathways in a reproducible manner, and (d) can be easily transferred to an autologous or even allogeneic host [5]. Tissue-specific stem cells originate from specific organs such as: brain, gut, lung, liver, bone marrow, and adipose tissue [6]. It is well known that these stem cells persist in adults; however they represent a rare population "hidden" amongst other cell populations [7]. ASC have a broad differentiation potential, but their ability to develop is limited compared to embryonic stem cells. They can be isolated from either bone marrow or adipose tissue. This population was initially thought to differentiate only to their tissue of origin; however, it has been shown that ASC have the capacity to differentiate into cells of mesodermal, endodermal, and ectodermal origin. Furthermore, they cross-lineage barriers and acquire the phenotype and biochemical properties of cells that are unique to other tissues [8–13]. Adipocytes develop from mesenchymal cells through a combination of transcriptional and nontranscriptional events that occur throughout human life. Adipocyte differentiation is a complex process accompanied by simultaneous changes in cell morphology, hormone sensitivity, and gene expression [5]. Although, for many years, ASC have been described as pre-adipocytes [14, 15], today they are appreciated as multipotent cells with a chondrogenic, neurogenic, and osteogenic potential [14–17]. Sedentary lifestyle and limited time for exercise have contributed to irregularities in body contour and excess adipocyte mass that is often resistant to the most strenuous exercise or weight loss efforts. The significant accumulation of subcutaneous fat among individuals in the United States and indeed world-wide in developed nations makes adipose tissue an abundant source of ASC. Approximately 400,000 liposuction procedures are performed in the United States each year, and these procedures yield anywhere from 100 mL to >3 L of adipocyte tissue [18]. Today, most of this lipoaspirate, which contains a significant amount of ASC with a wide range of therapeutic potential, is discarded.

10.2 Biomolecules and Adipose Stem Cells

Biomolecules refer to the biological materials which serve as the structural integrity of tissue-engineered constructs and regulate their components. The main components of biomolecules are the following cellular factors: growth, differentiation, angiogenic, pro-inflammatory, and gene modulated. The specific factors to be used for each tissue-engineered construct can be provided either exogenously or by local or systemic delivery. Adipose tissue is a dynamic "player" in endocrine physiology and serves as a source of cytokine secretion. In the clinical setting, it has been shown that individuals with large volumes of adipose tissue are more likely to have increased levels of pro-inflammatory cytokines such as interleukin (IL) 6, IL-8, and tumor necrosis factor alpha (TNF-α). Furthermore, adipose tissue expresses hematopoietic growth factor and macrophage colony-stimulating factor (M-CSF), whose expression can lead to adipose tissue volume expansion [19].

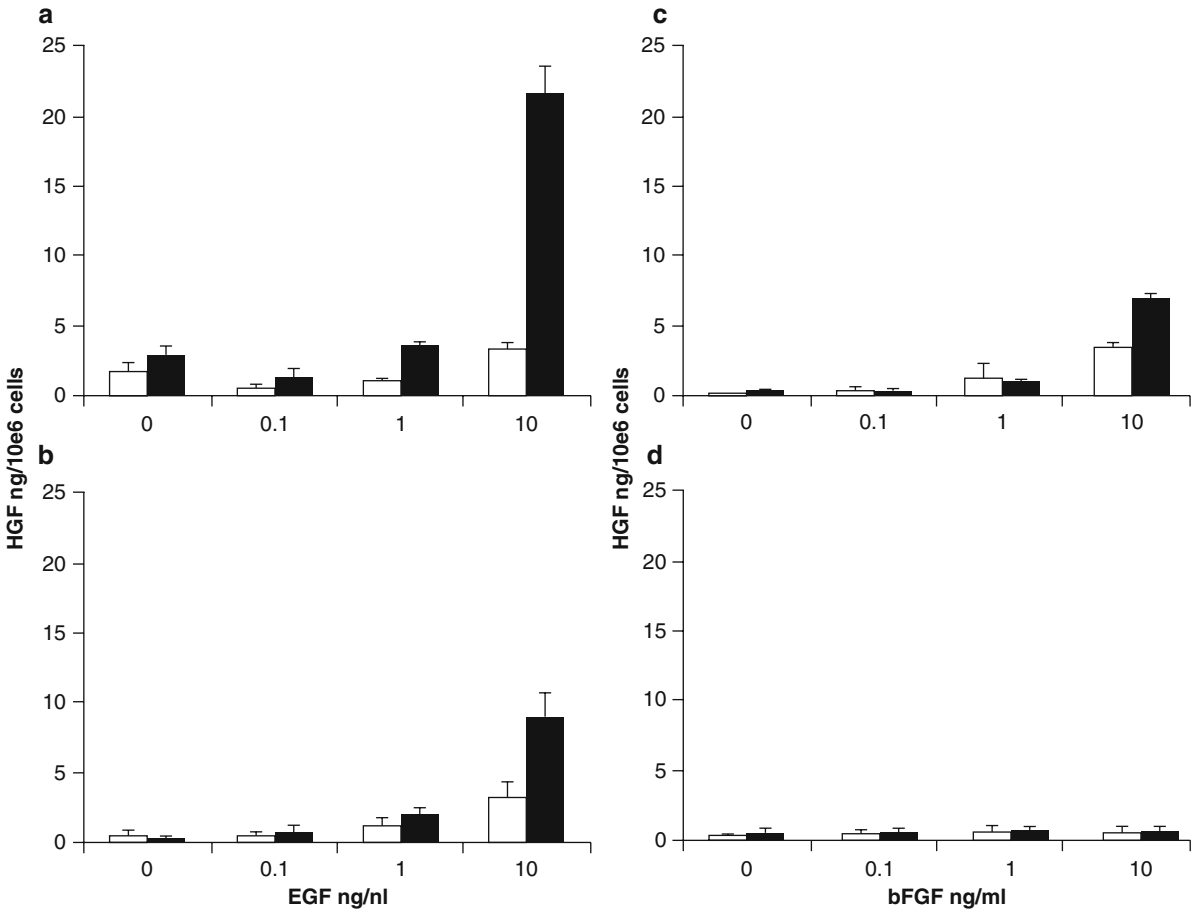
ASC are multipotent and can potentially differentiate in various pathways in response to growth factors and environmental agents [20]. There is evidence that ASC can promote tissue recovery through the delivery and localized secretion of cytokines. Recent in vivo studies support this hypothesis. Intravenous infusion of ASC improved recovery of limb function in mice following ischemic injury [21]. The positive effects of ASC in ischemia are most likely secondary to their ability to secrete angiogenic cytokines, such as hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF).

In this chapter the authors reviewed the endocrine function and cytokine profile of ASC, and focused on elucidating the basic principles, as well as interactions, between adipose stem cells and cytokines, adipokines, or biomolecules in general.

10.2.1 Angiogenic Factors

10.2.1.1 Hepatocyte Growth Factor (HGF)

The role of implanting ASC into ischemic cardiac tissue as a means to increase angiogenesis is an emerging therapeutic approach [22, 23]. Most of the



[AU6]

Fig. 10.1 Hepatocyte growth factor (*HGF*) secretion. The secretion of HGF was determined by ELISA on conditioned medium from undifferentiated (**a**, **c**) and adipocyte-differentiated (**b**, **d**) ASC following exposure to epidermal growth factor (*EGF*) (**a**, **b**) or basic fibroblast growth factor (*bFGF*) (**c**, **d**) in the absence (*white bars*) or presence (*solid bars*) of varying concentrations of 2-sodium ascorbic acid. The values represent the mean (ng/10^6 cells) ± S.D. of $n = 3$ ASC donors (Reprinted with permission from the publisher from Kilroy et al. [19])

clinical studies have used bone marrow cells which are only available in limited quantities and cannot be easily isolated. There are data to support that ASC secrete HGF, thus representing a potential source for cells to be utilized in cardiovascular cell therapy [19, 24, 25]. In vitro studies have depicted a link between ASC-derived HGF and physiologic or pathologic processes. In particular, secretion of HGF by ASC has been shown to have a positive effect on tubule formation by vascular endothelial cells. This action was found to be independent of VEGF [26]. Unfortunately, Rahimi et al. showed that HGF secreted by ASC promoted the proliferation of mammary tumor epithelial cells [27]. Kilroy et al. reported the constitutive and inducible secretion of HGF by ASC in vitro. The authors showed that this property was dependent on the level of ASC differentiation. In particular, the adipocyte-differentiated ASC appear to lose their responsiveness to basic fibroblast growth factor (b-FGF) and failed to induce HGF expression. On the other hand, treatment of undifferentiated ASC with either b-FGF or EGF was associated with increased levels of HGF release. Finally, it appears that the addition of ascorbic acid increased the increased HGF secretion by a factor of twofold or greater (Fig. 10.1) [19].

In a similar manner, Rehman et al. reported the secretion of HGF by human ASC in significant

amounts (12,280 ± 2,944 pg/10^6 cells). In order to assess potential in vivo viability and function, the authors transduced ASC, with a GFP-expressing adenovirus to facilitate tracking into mice limbs. One week after injection, 28 ± 2% of injected cells could be identified on serial sections of the muscle [25].

10.2.1.2 Vascular Endothelial Growth Factor (VEGF)

Vascular endothelial growth factor (VEGF) promotes neovascularization during embryonic development, subsequent to tissue injury, following exercise, and under ischemic conditions, in general. It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate. VEGF is a subfamily of growth factors, specifically the platelet-derived growth factor family of cystine-knot growth factors. They are important signaling proteins involved in both vasculogenesis (the *de novo* formation of the embryonic circulatory system) and angiogenesis (the growth of blood vessels from preexisting vasculature). While secretion of VEGF by bone marrow stem cells has been documented [28], Rehman et al. [25] showed that ASC represent a source of VEGF, as well. The authors reported that over a 72-h period in basal medium with 5% fetal bovine serum and no additional growth factors under normoxic conditions, ASC secreted significant amounts of VEGF (1,203 ± 254 pg/10^6 cells). Interestingly, when ASC were cultured in hypoxic conditions, there was a fivefold increase in the secretion of VEGF from 1,203 ± 254 to 5,980 ± 1,066 pg/10^6 cells ($p = 0.0016$, paired t-test, $n = 7$). The property of ASC to react to a stimulus such as hypoxia shows that they can adapt to the environment into which they are placed (ischemic myocardium), by increasing the production of VEGF in response to ischemia and thus, induce neovascularization.

10.2.2 Hematopoietic and Pro-inflammatory Factors

One of the most clinically relevant properties of bone marrow-derived mesenchyme is the ability to provide long-term hematopoietic support. ASC appear to have a similar level of hematopoietic cell expansion when compared with bone marrow-derived stroma cells. In order to assess their ability toward hematopoietic differentiation, Kilroy et al. [19] used purified CD34p Linneg cells to initiate long-term culture assays on ASC. After either 3 or 5 weeks, the cultures were examined to assess whether clonogenic myeloid cells (CFC) had been maintained. Although hematopoiesis was present in the 3-week cultures; by 5 weeks, less clonogenic progenitors had been maintained. Those preliminary results suggested that ASC can preserve hematopoiesis in vitro, especially in the short-term period. In order to directly compare the hematopoiesis potential of ASC and marrow-derived cells, the authors subsequently established long-term culture assays. Their results suggest that marrow-derived stroma cells provided more efficient long-term support for primitive progenitors. Although ASC were less efficient than marrow cells, they still exhibited some true hematopoietic ability. When the authors exposed ASC to lipopolysaccharide (LPS), which is an agonist for bone marrow stromal cell cytokine induction, the level of secreted IL-6 and IL-8 increased. More specifically, both IL-6 and IL-8 reached maximal mean levels of 7,845 and 6,506 pg/mL conditioned medium, respectively, after 24 h of LPS exposure. Similarly, the hematopoietic cytokines: macrophage colony-stimulating factor (M-CSF) and granulocyte–macrophage colony-stimulating factor (GM-CSF) reached maximal mean levels of 976 and 52 pg/mL, respectively, at 24 h. TNF-α however, reached its peak mean level of 112 pg/mL after 8 h of LPS exposure. IL-7 and the pro-inflammatory cytokine IL-11 were low. They displayed a significant induction by ELISA, reaching maximal mean levels 24 h after LPS exposure of 3.4 and 12.7 pg/mL, respectively (Fig. 10.2).

Consistent with the ELISAs, the steady-state levels of mRNAs for representative cytokines were elevated within 4 h following LPS exposure based on RT-PCR. IL-1a, IL1b, and IL-12 protein were not detected in the conditioned medium from undifferentiated ASC following LPS exposure. The data produced by this study indicate that ASC may have clinical value for the patient population undergoing hematopoietic stem cell transplantation following high-dose chemotherapy. Conclusively, there is potential of co-infusing ASC with hematopoietic stem cells as a means to optimize

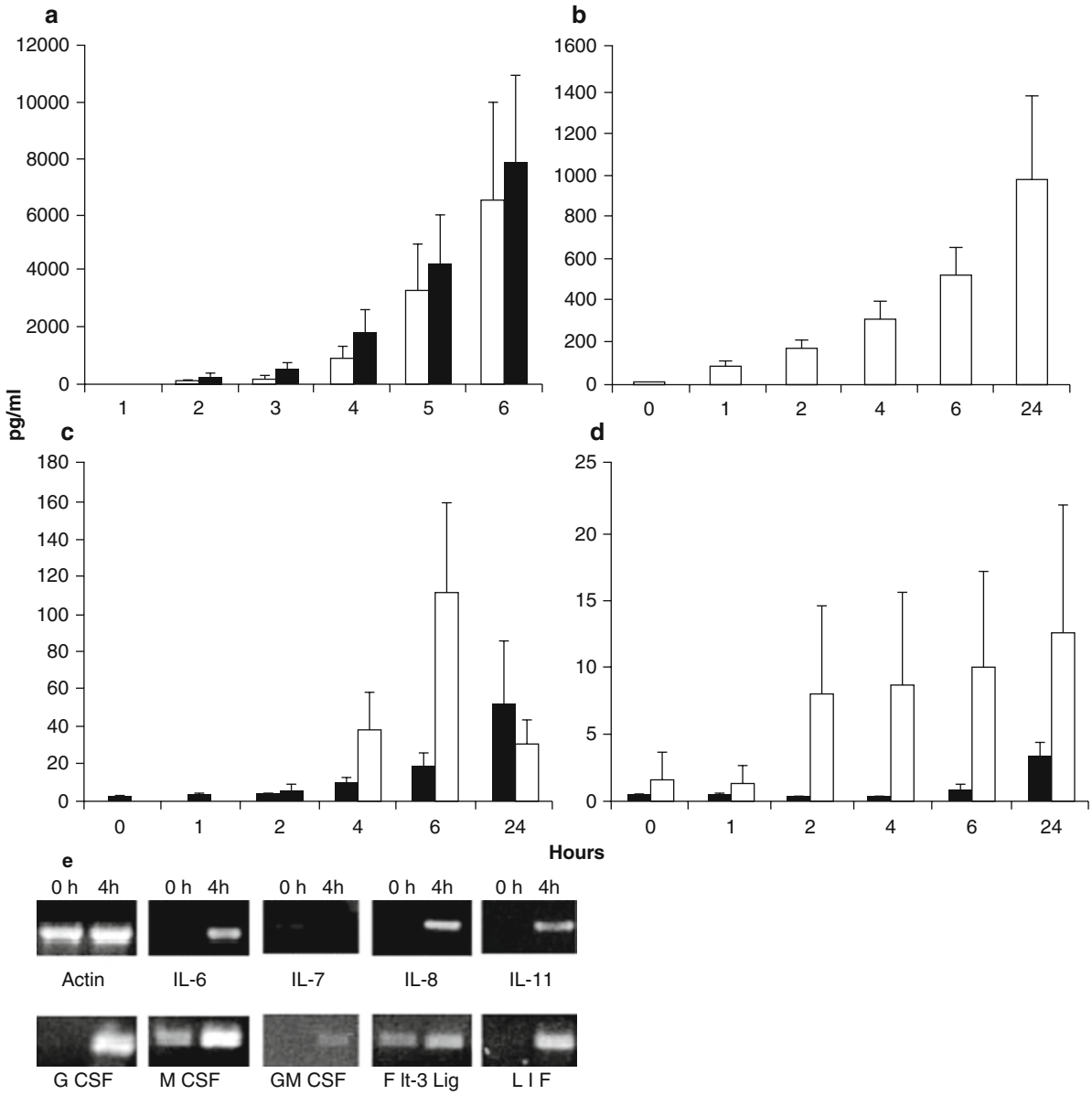


Fig. 10.2 Pro-inflammatory and hematopoietic cytokine secretion. The conditioned medium from undifferentiated ASC was assayed for secretion of selected cytokines at varying times following exposure to LPS (100 ng/mL) for periods of 0–24 h; (**a**) IL-6 (*solid bar*) and IL-8 (*clear bar*); (**b**) M-CSF; (**c**) GM-CSF (*clear bar*) and TNF (*solid bar*); (**d**) IL-7 (*clear bar*) and IL-11 (*solid bar*). The values represent the mean (pg/mL) ± S.E.M. of $n = 6–8$ ASC donors. (**e**) The mRNA levels of selected cytokines in ASC from a representative donor were assayed by PCR analysis following exposure to LPS (100 ng/mL) for 0 or 4 h (Reprinted with permission from the publisher from Kilroy et al. [19])

t1.1 **Table 10.1** Current possible biomolecules used in adipose tissue engineering

t1.2 Types of biomolecules	Properties
t1.3 t1.4 Fibroblast growth factor-2 (FGF-2)	Promotes chondrogenic and inhibits osteogenic differentiation of ADSCs [29]
t1.5 t1.6 Platelet-derived growth factor (PDGF)-AB	Proliferation potential on human adipose-derived stem cells and human dermal fibroblasts [30]
t1.7 t1.8 Transforming growth factor (TGF)-beta1	Proliferation potential on human adipose-derived stem cells and human dermal fibroblasts [30]
t1.9 Vascular endothelial growth factor (VEGF)	Improves implant biocompatibility [31]
t1.10 t1.11	Promotes capillary formation in adipose stem cell containing tubular scaffolds [32]
t1.12 Granulocyte/macrophage colony-stimulating factor	Angiogenesis-related cytokine secreted by ADSCs [33]
t1.13 Stromal-derived factor-1alpha	Angiogenesis-related cytokine secreted by ADSCs [33]
t1.14 Hepatocyte growth factor	Angiogenesis-related cytokine secreted by ADSCs [33].

250 recovery of normal blood cell production and subse-
251 quently restore immune function.
252 The possible biomolecules used in adipose tissue
[AU2]
253 engineering are shown in Table 10.1.

254 10.3 Conclusions

255 The evolving field of producing organs from the basic
256 life unit, a cell, can potentially provide a unique solu-
257 tion to the aforementioned problems. The ability of
258 ASC to secrete several biologic factors plus evidence at
259 a basic science level lends way to ASC playing a major
260 role in tissue engineering and organ regeneration.

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