Animal Models for Adipose Tissue Engineering

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ABSTRACT

There is a critical need for adequate reconstruction of soft tissue defects resulting from tumor resection, trauma, and congenital abnormalities. To be sure, adipose tissue engineering strategies offer promising solutions. However, before clinical translation can occur, efficacy must be proven in animal studies. The aim of this review is to provide an overview of animal models currently employed for adipose tissue engineering.

INTRODUCTION

THOMAS KUHN, HISTORIAN AND PHILOSOPHER OF SCIENCE, states that every scientific age possesses its paradigms, theories nearly universally regarded as true that form the framework for ongoing scientific investigation.¹ A currently dominant paradigm is that animal models are necessary for medical progress. The intent of animal models is to closely represent human tissue and physiology. Galen of Pergamon (130 AD–200 AD), one of Western society's earliest experimental anatomists and physiologists, conducted research primarily on Barbary apes and pigs (human dissection was prohibited in Greece and Rome at the time). By the 18th and 19th centuries, the use of animal models matured from an uncommon practice into scientific necessity. The Office of Technology Assessment estimates that 17–23 million animals are used in the United States for research every year.² Extrapolation of information gained from animal models to the human can be fairly accurate or grossly in error.

Adipose tissue engineering has been an area of relatively intense research for the past decade. The clinical impetus is understandably large, ranging from trauma, disease, and congenital abnormalities.³ Before newly developed products can be applied in human patients, their final efficacy must be proven in animal studies. Individual investigators globally have employed a host of animal models to develop, assess, and validate strategies. Most investigators

have relied on small animal models (mouse and rat) due to costs and the short experimental time constants. Approximately 97% of all lab animals in the United States are mice and rats.⁴ A few investigators have begun to utilize pre-clinical large animal models in adipose tissue engineering. This is a step the field in general needs to take if strategies are to be translated to the clinic.

Although a wealth of information has been generated in the area of adipose tissue engineering, the diversity of animal models and experimental variation within the same species and laboratory render direct comparison of data impossible. Factors that need to be considered in an animal model include technical requirements of implanting the construct, availability of the animal, costs, and ethical considerations. We review the animal models that have been used to date in adipose tissue engineering, highlighting realized limitations, experimental variation, and, when possible, correlation of data-driven conclusions.

REVIEW METHODS AND DEFINITIONS

In this review, adipose tissue engineering refers to a strategy consisting of a biomaterial (natural, synthetic, or hybrid) possessing cells and/or bioactive factors. The biomaterial can be utilized as a cell adhesive or structural scaffold and/or as a delivery vehicle for bioactive factors. Free fat grafting is not considered here. The PubMed

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database was mined from 1/1/1998 to 9/30/2007. Other sources of information include conference proceedings and personal communication. Only *in vivo* studies are included in this review. It is acknowledged that there has been a wealth of *in vitro* studies reported in the diabetes, obesity, physiology, reconstructive surgery, and regenerative medicine literature.

All implantation times are presented in units of weeks. When studies reported implantation times in units of months, the values were converted to weeks based on 4 weeks/month. Several studies did not include detailed information regarding the animal models (e.g., strain, gender, and weight); these studies are incorporated in this review, with missing data labeled as "unknown."

Nomenclature of adipose-derived cells (ADCs) is not consistent in the literature. Investigators refer to cells as preadipocytes, adipose-derived stem cells, adipose-derived stromal cells, stromal vascular fraction, and ADCs despite isolation methodologies being apparently identical or very similar. Further, it is unclear whether some investigators use a homogeneous or heterogeneous population of cells. In this review, we refer to cells used to seed scaffolds as ADCs rather than attempting to delineate between the cell types.

METAANALYSES

Tables 1–3 present a summary of the small and large animal models employed to date. In approximately the past decade, there have been 41 adipose tissue engineering studies employing animal models. The majority of studies employ small animal models, with 22 using mouse and 15 using rat models. The remaining studies employ various preclinical large animal models. Figure 1 demonstrates that the cumulative number of published *in vivo* adipose tissue studies possesses a constant rate of 4.4 instances/year ($R^2 = 0.96$). One interpretation of these data is that the adipose tissue engineering field is consistently being investigated by the same few groups in the United States, Japan, Europe, and Australia based on the fact that the curve is essentially linear. Additional teams of investigators are required to join the field in order to significantly alter its trajectory. We further analyze the data in Tables 1–3 in terms of strains, gender, and implant time, location, and configuration.

Strains

Figure 2 illustrates the distribution of mouse and rat strains employed. Of the mouse models employed, the majority (17/23) are immunodeficient strains, including nude Bagg Albino (BALB/c-nu, 5/23), Naval Medical Research Institute (NMRI-nu) (4/23), severe combined immunodeficiency (SCID, 3/22), and KSN (1/22). The exact strains of an athymic (2/23) and athymic nude (2/23) mouse model were not identified in four studies. The remaining mouse studies employed BALB/c (3/23) and C57BL/6 (3/23)

mice. There are literally thousands of mouse strains available for research; The Jackson Laboratory alone possesses over 3100 mouse strains.⁵ No specific rationale is provided by investigators as to the strain selected. However, review of investigators' past publications suggests that a laboratory's historical use of a particular strain dictates future selection of a particular strain. Immunodeficient strains are selected if xenogeneic or transgenic cells are to be transplanted into host mice.

Of the rat models employed, an equal number of studies employed either Sprague-Dawley (SD, 4/15) or Wistar (WIS, 4/15) strains. The Lewis (LEW) strain was employed to a slightly higher extent (5/15). In the remaining two studies, one used an immunodeficient strain (Ratnude, RNU) and the other study did not identify a strain.

Two large animal models have been employed. One study used domestic sheep. The remaining studies used porcine models. No strain was mentioned other than the term of domestic pig. The designation of domestic pig excludes minipigs and micropigs, but can include different breeds and strains possessing different adipose tissue content and deposition patterns.

Gender

It is of critical importance to note the gender of animals employed since gender-specific parameters may confound data interpretation. For instance, male rats and mice grow larger than their female counterparts. In addition, it has been known for some time that female rats possess hormonal conditions that affect adipogenesis. For instance, it has been demonstrated that estrogens behave as proadipogenic hormones⁶ and that ovarian factors affect the preadipocyte differentiation.⁷ The gender of mice used was even, with 6/23 and 6/23 studies using male and female mice, respectively. Surprisingly, half the studies (12/23) did not document the gender of mice employed. One study used a mixture of genders.⁸ The majority of the rat studies used male rats (8/15). Three studies used female rats. The remainder of the studies (4/15) did not document the gender of the rats employed. For the large animal models, female sheep and male pigs were used.

Implantation time

The development of clinically translatable strategies necessitates investigation at time points that are clinically meaningful. This typically correlates to implantation times on the order of 6 months to 1–2 years. Implantation time in tissue engineering constructs is perhaps even more important because of the tendency for *de novo* tissue growth to resorb over a rather short time period. Compounding this issue for adipose tissue engineering is the fact that clinical fat grafts tend to resorb. In general, small animal models are utilized for proof-of-principle or short-term studies. Hence, the maximum mouse study time ranged from 2 weeks

TABLE 1. *In vivo* ADIPOSE TISSUE ENGINEERING STUDIES USING MOUSE MODELS

Strain	Gender	Implant location	Biomaterial scaffold	Seeded cells	Bioactive factors	Implantation time (weeks)	Refs.
BALB/c	F	Dorsal subcutis	Matrigel	None	FGF-2 in gelatin microspheres	6	15
	F	Dorsal subcutis	Matrigel	None	FGF-2 in gelatin microspheres	2, 4, 6, 12, 15	34
	F	Dorsal subcutis	Collagen	Hu ADCs	FGF-2 in gelatin microspheres	6	58
	M	Vascular pedicle in groin	Polycarbonate and silicone chambers filled with Matrigel or PLGA	None	None	2, 4, 6	9
C57BL/6	M	Vascular pedicle in groin	Silicone chamber filled with Matrigel	None	FGF-2	6	10
	M	Vascular pedicle in groin	Silicone chamber filled with collagen	None	FGF-2 in gelatin microspheres	6	11
KSN	F	Dorsal subcutis	Matrigel	None	FGF-2	1, 2	14
BALB/c-nu	M	Dorsal subcutis	Fibrin	Hu ADCs	None	4, 8, 24, 36	23
	F	Cervical subcutis	PLGA	ADCs	None	4, 8	58
	M/F	Dorsal subcutis	Collagen	ADCs from GFP transgenic mice	None	8	8
	Unknown	Cervical subcutis	PLGA	Rabbit MSCs	None	2	59
				differentiated into adipocytes			
NMRI-nu	Unknown	Thoracic subcutis	Matrigel	3T3-F442A	FGF-2	2, 5, 10	60
	Unknown	Scapular subcutis	Collagen	Hu ADCs	None	3, 8	61
	Unknown	Scapular subcutis	Collagen	Hu ADCs	None	3, 8, 12	62
	Unknown	Scapular subcutis	Hyaluronan	Hu ADCs	FGF-2	3, 8, 12	63
	Unknown	Dorsal subcutis	PGA	3T3-L1	None	1, 3	18
SCID	M	Dorsal subcutis	Gelatin	Hu ADCs	None	4	64
	Unknown	Vascular pedicle in groin	Silicone chamber filled with Matrigel	Hu ADCs or fat	FGF-2	6, 9	36
	Unknown	Dorsal subcutis	PEGDA	Hu MSCs	None	4	65
Unknown athymic	Unknown	Dorsal subcutis	PGA/PLLA scaffolds filled with fibrin	Hu ADCs	FGF-2	6	66
	Unknown	Dorsal subcutis	Fibrin	Hu ADCs	FGF-2	6	37
Unknown athymic nude	Unknown	Dorsal subcutis	Fluoropolymers (U48 and P54)	Hu ADCs	None	4	67
	Unknown	Dorsal subcutis	PEG and collagen	Hu MSCs	None	4	68
				differentiated into adipocytes			

BALB, Bagg Albino; SCID, severe combined immunodeficiency; NMRI-nu, Naval Medical Research Institute-nude; ADCs, adipose-derived cells; FGF-2, fibroblast growth factor-2; PEG, poly(ethylene glycol); PEGDA, PEG diacrylate; PGA, poly(glycolic acid); PLLA, poly(L-lactic acid); PLGA, poly(L-lactic-co-glycolic acid); GFP, green fluorescent protein; Hu, human; MSCs, mesenchymal stem cells.

TABLE 2. *IN VIVO* ADIPOSE TISSUE ENGINEERING STUDIES USING RAT MODELS

Strain	Gender	Implant location	Biomaterial scaffold	Seeded cells	Bioactive factors	Implantation time (weeks)	Refs.
LEW	M	Dorsal subcutis	PLGA	Isogenic ADCs	None	2,5	42
	M	Dorsal subcutis	PLGA	Isogenic ADCs	None	4, 8, 12, 20, 28, 36, 48	22
	M	Dorsal subcutis	Proprietary J&J materials	Isogenic ADCs	None	1, 2	17
SD	M	Dorsal subcutis	Collagen-chitosan	Isogenic ADCs	None	1, 2	16
	Unknown	Cranial subcutis	PGA	Isogenic ADCs	None	4, 8	69
	F	Dorsal subcutis	None	Autologous inguinal fat grafts	IGF-1, FGF-2, and insulin in PLGA/PEG microspheres	12	38
F	F	Abdominal subcutis	None	None	IGF-1 and insulin in PLGA/PEG microspheres	4	70
M	M	Vascular pedicle in groin	Polycarbonate chambers filled with PLGA	Existing fat	None	6	40
WIS	M	Vascular pedicle in groin	Polycarbonate chamber filled with PLGA	None	None	2, 4, 6, 8	13
	M	Fibrous capsule in rectus abdominus muscle	Fibrin	Autologous ADCs	None	2, 4, 12, 24, 48	21
	M	Dorsal subcutis	None	None	IGF-1, FGF-2, and insulin in styrenated gelatin microspheres	2, 4, 6	39
F	F	Inguinal fat pad defects	Collagen-PP	Syngeneic ADCs	FGF-2 in gelatin microspheres	2, 4, 6	33
Unknown	Unknown	Fibrous capsule in rectus abdominus muscle	Fibrin	Autologous ADCs	None	4, 12, 24, 48	20
RNU	Unknown	Vascular pedicle in groin	Silicone chamber filled with Matrigel and PGA	None	FGF-2	4, 8, 12, 16, 20	12
Unknown	Unknown	Scapular subcutis	Hyaluronic acid based (HY/ADD11)	Autologous ADCs	None	12	71

LEW, Lewis; SD, Sprague-Dawley; WIS, Wistar; RNU, ratnude; ADCs, adipose-derived cells; FGF-2, fibroblast growth factor-2; I&J, Johnson & Johnson; PEG, poly(ethylene glycol); PGA, poly(glycolic acid); PLLA, poly(L-lactic acid); PLGA, poly(L-lactic-co-glycolic acid); PP, polypropylene; IGF, insulin growth factor-1.

TABLE 3. *IN VIVO* ADIPOSE TISSUE ENGINEERING STUDIES USING LARGE ANIMAL MODELS

Descriptor	Gender	Implant location	Biomaterial scaffold	Seeded cells	Bioactive factors	Implantation time (weeks)	Refs.
SHEEP							
Domestic	F	Cervical subcutis	RGD-derivatized alginate	Autologous ADCs	None	4, 12	27
PIG							
Unknown	Unknown	Vascular pedicle in breast region	Polycarbonate chamber	Autologous fat	None	12	28
Unknown	Unknown	Ear subcutis	Hyaluronan-based gels (HYADD3, HYADD4)	Autologous ADCs	None	6	29
Domestic	M	Vascular pedicle in groin	Silicone chamber filled with collagen: chitosan	Autologous ADCs	None	2, 6, 8	

ADCs, adipose-derived cells; RGD, Arg-Gly-Asp.

to 36 weeks, with a mean $\pm$ SEM of 8.0 ± 1.5 weeks (Fig. 3). Half of the studies used only one time point, thereby preventing any kinetic study of tissue formation. Similarly, the maximum rat study time ranged from 2 weeks to 48 weeks, with a mean $\pm$ SEM of 15.7 ± 4.5 weeks (Fig. 3). All but three studies used multiple time points to investigate tissue kinetics.

Large animal models are typically utilized in order to overcome two primary limitations with small animal models, namely, assessing clinically sized manipulations (e.g., defects and implants) and observing the model long term. However, all large animal studies were rather short term (6 and 12 weeks). This points to the fact that the field

FIG. 1. Cumulative total of *in vivo* studies employing mouse, rat, and large animal models from 1/1/1998 to 9/30/2007.

FIG. 2. Distribution of mouse (A) and rat (B) strains employed.

FIG. 3. Distribution of implant time.

of adipose tissue engineering is at the vernal stage of developing large animal models.

Implant location

Figure 4 illustrates the anatomical locations where engineered adipose tissue constructs were implanted in the mouse and rat models. The preponderance of constructs is placed in subcutaneous areas largely because of ease of surgical implantation, harvest of tissue samples, and interpretation of subcutaneous histology. In the mouse models, the majority of constructs (13/23) were placed in the dorsal subcutis. Other subcutaneous locations included the cervical (2/23), scapular (3/23), and thoracic (1/23) regions. Four studies used a vascular pedicle in the groin using either femoral vessels⁹ or superficial inferior epigastric vessels.^{10,11} The vascular pedicle with an angiogenic network supports the construct. In addition, the vascular pedicle permits transfer of the construct to another anatomical location at a later time using conventional microsurgical techniques. That is, *de novo* adipose tissue can be generated at one site and then transferred to a second site to repair a tissue deficit.

The anatomical location for the rat models was also primarily in the subcutaneous area (9/15), including dorsal (6/15), abdominal (1/15), cranial (1/15), and scapular (1/15) subcutis regions. Two studies implanted constructs in pre-formed fibrous capsules located in the rectus abdominus muscle, and one study implanted constructs in inguinal fat pad defects. Finally, three studies used a vascular pedicle in the groin using superficial inferior epigastric vessels in a flow through configuration¹² or femoral vessels in an arteriovenous shunt configuration.¹³

FIG. 4. Distribution of implant locations in the mouse and rat models. Summation of percentages does not equal 100 due to rounding. AS, abdominal subcutis; CS, cervical subcutis; CrS, cranial subcutis; DS, dorsal subcutis; IFP, inguinal fat pads; RAM, rectus abdominus muscle; SS, scapular subcutis; TS, thoracic subcutis; VP, vascular pedicle in groin.

For the large animal models, two studies were implanted in subcutaneous areas. Specifically, the sheep model utilized the cervical subcutis and a pig model utilized the ear subcutis. The remaining pig studies employed a vascular pedicle located at the groin or breast.

Implant configuration

A conventional tissue-engineered construct may possess (a) a scaffold for cell seeding and/or initial shape and volume, (b) seeded autologous, xenogeneic, or engineered cells, (c) bioactive factors to mediate vascularization and/or tissue growth, and (d) a vascular pedicle to provide vascularization. All three components may be incorporated or any combination thereof. Constructs that do not possess seeded cells rely on the recruitment of local precursor and/or stem cells. If bioactive factors are a component of the construct, it is desired that factors be released locally rather than systemically. Even then, oncologic predisposition of patients may prevent

the use of local bioactive factors if the strategy is translated to the clinic. If bioactive factors are not provided, constructs rely on factors released by the wound healing cascade and local cells and extracellular matrix (ECM). Collectively, the studies used a wide variety of natural and synthetic scaffolds (Tables 1–3) possessing different degradation kinetics, biomechanical properties, and immune responses.

In the mouse models, 13/23 studies implanted constructs consisting of a scaffold seeded with cells without supplementing with exogenous bioactive factors. In addition, 5/23 studies scaffolds and bioactive factors, but no cells. A few studies (4/23) incorporated all three elements of a construct. One study used only a scaffold and a vascular pedicle to induce adipogenesis.

Similar to the mouse models, the majority (8/15) of the rat models implanted constructs consisting of a scaffold seeded with cells without supplementing with exogenous bioactive factors. Only one study incorporated all three elements of a construct. In addition, two studies used a scaffold alone and a vascular pedicle to induce adipogenesis. Other construct configurations included scaffold and bioactive factors (1/15), cells and bioactive factors (1/15), and bioactive factors alone (2/15).

In all large animal models, scaffolds seeded with autologous cells were implanted. No bioactive factors were used.

DISCUSSION AND TRENDS

As illustrated in the 42 *in vivo* studies outlined in Tables 1–3, a consensus animal model for use in adipose tissue engineering currently does not exist. One of the advantages of a rodent model is the reproducibility of the model, due to minimal genetic variance. However, even different rodent models employed cannot be directly compared, due to the difference between strains. For example, WIS rats typically grow larger than LEW and SD rats, and, as a result, the mass and growth rate of adipose tissue varies. In addition, although LEW rats were derived from WIS stock, they possess high insulin and growth hormone levels, resulting in obesity when on a high-fat diet. BALB/c and C57BL/6 mouse strains are nonimmunodeficient, but possess different amounts of adipose tissue. Female and male BLAB/c mice possess 22.2% and 20.2% adipose tissue, respectively.⁵ Female and male C57BL/6 mice possess 17.7% and 19.8% adipose tissue, respectively.⁵ Despite the lower percentage of adipose tissue in C57BL/6 mice, they are highly susceptible to diet-induced obesity. RNU rats are immunodeficient and permit transplantation of xenografts, but the decreased T cell populations and increased natural killer and macrophage cell populations results in wound healing kinetics different from nonimmunodeficient LEW, SD, and WIS rats. Similarly, the level of immunodeficiency varies among the BALB/c-nu, SCID, NMRI-nu, and KSN immunodeficient mouse strains. For instance, SCID mice possess combined immunodeficiency affecting T and B cell development, but

they do possess a thymus and normal natural killer and macrophage cell populations. On the other hand, BALB/c-nu, NMRI-nu, and KSN lack a thymus, affecting T cell development, but B cell populations are normal. These variations affect wound healing kinetics and prevent direct comparison of adipogenesis. We have personally refrained from using a mouse model based solely on the fact that defect sizes are entirely too small to observe any limits of revascularization on adipogenesis. For proof-of-principle studies, we recommend the use of an inbred strain of rat (like LEW) so that transplantation between donors and recipients are isogenic. With the wealth of antibodies on the market, immunohistochemical studies that once were conducted in mice are readily available for rat models.

At this point, it is unclear to what extent differences in fat composition, insulin levels, wound healing kinetics, and other physiological factors confounded interpretation of *de novo* adipogenesis in the studies presented in Tables 1–3 and, more importantly, comparison of studies possessing different animal strains. For instance, Toriyama *et al.*¹⁴ and Tabata *et al.*¹⁵ conducted the same study (dorsal subcutis implantation, Matrigel scaffold possessing fibroblast growth factor-2 [FGF-2], and no cell seeding) but used different female mouse strains (KSN vs. BALB/c, respectively). Both studies demonstrated *de novo* adipogenesis, but it is unclear if differences in the amount of adipogenesis are related to the differences in mouse strains. In human-based studies, one obtains a body mass index and a measure of insulin levels in order to provide a baseline for comparing any adipose-related study. Similar baseline metrics are required in adipose tissue engineering.

The majority of the studies (38/42) presented in Tables 1–3 are short-term studies (<24 weeks). Of these, approximately half (20/38) conducted kinetic studies where multiple time points were utilized. Since the hemostasis, inflammation, and proliferative phases of wound healing occur at immediate-1 day, 1–4 days, and 4 days–3 weeks, respectively, it may be important to design experiments to include time points beyond these time frame so that wound healing kinetics will not confound interpretation of *de novo* adipogenesis. Several studies did not possess time points beyond 3 weeks.^{14,16–19} Although *in vitro* differentiation of preadipocytes to adipocytes requires several weeks to occur in a controlled environment, *in vivo* adipogenesis is observed to occur in as little as 2 weeks.^{16,17} All 42 studies demonstrate that adipogenesis can occur. However, studies involving longer time points (>24 weeks) are required to begin to address tissue permanence mechanisms. Four studies assessed adipose tissue engineering for time points >24 weeks.^{20–23} Results from the four long-term studies have been mixed: from complete resorption >5 weeks,²² to 50% reduction after 4 weeks followed by persistence until at least 9 months,²³ and to stable persistence for 1 year.^{20,21} Differences in the study results may be attributed to the varied scaffold materials, animal models, and/or the microenvironment seeded cells were placed. As the progress

in adipose tissue engineering translates into the clinic, tailoring techniques toward different anatomical site-specific applications will be necessary. For example, the biomechanical environment of postoncologic reconstructed breast tissue will be different from the environment of a reconstructed face. Animal models that present anatomical and biomechanical environments similar to man will be essential. The field of adipose tissue engineering is just now delving into the use of large animal models, so it remains to be determined which specific models are the most appropriate. That being said, large animal models have the advantage of simulating the anatomical, physiological, and biomechanical environment of humans far better in comparison to rodents. For example, humans are about 2000–3000 times larger and live about 30–50 times longer than small rodents. Given the lifelong turnover of the bodies, humans undergo about 100,000 more cell divisions in a lifetime.²⁴ Mice possess a basal metabolic rate seven times higher than that in humans.²⁵ On the other hand, the similarity between human and porcine anatomy and physiology spans the development of oocytes to anatomy and function of the adult body.²⁶ Two large animal models have been tested to date: sheep²⁷ and pig.^{28,29} Pigs appear to be the most appropriate model. Pigs are extensively used as a model to train surgeons in various complex surgical techniques due to the anatomical similarity. In addition, pigs are employed as a plastic and reconstructive surgery model due to similarities of porcine and human skin. Though extensive work has not been done in comparison of stem cells of pig and human, neural stem cells of pig and human were observed to be behaving the same way with respect to growth conditions, growth factor requirements, and patterns of differentiation.²⁶

Despite the disparate differences in the studies, several general concepts can be gleaned when they are viewed collectively. For instance, the studies clearly demonstrate that adipose tissue can be generated, at least short term, if the correct environment is created. However, it is not clear whether an optimum biomaterial exists. The studies pre-

sented in Tables 1–3 demonstrate that natural and synthetic hydrogels, polymer sponges, and nonwoven fiber meshes all support adipogenesis. These materials have been thoroughly reviewed with respect to adipose tissue engineering.^{30,31} The materials have largely been employed as a cell adhesive and structural component of a construct. No biomaterial optimization beyond manufacturing has occurred to any large extent. For example, the mechanical properties of the biomaterials have not been investigated. Presumably, there will be an influence of mechanical modulation of the engineered adipose tissue constructs. One study did compare the mechanical properties of a PEG hydrogel to human adipose tissue.³² Further such studies are required to determine the desired biomechanical properties of specific adipose tissue engineering strategies. In addition, the influence of scaffold size on tissue growth has not been studied, nor has the relation between scaffold degradation and adipogenesis. Moreover, only scaffold architecture at the micron dimension has been modified. Yet, cells interact with ECM proteins and surfaces at the nanometer-length scale. Hence, the effect of nanotexture on adipogenesis requires investigation.

Another overarching concept, and perhaps not surprising, is that there is a close association between adipogenesis and angiogenesis. It is for this reason that numerous investigators employed FGF-2^{10–13,15,19,29,33–39} or vascular pedicles^{9–13,28,36,40} in their construct designs. Adipose tissue is highly vascular, possessing resting values of blood flow and capillary filtration coefficients two to three times higher than those in skeletal muscle. This suggests that a rich vascular network is crucial to support the demands of an adipose construct. It is perhaps the most critical factor limiting the size, maintenance, and quality of the construct. The close inter-relationship between adipose tissue and vascular tissue *in vivo* has been demonstrated on a number of levels: embryologic, developmental, anatomic, and metabolic. It is important to note that angiogenesis precedes adipogenesis.⁴¹ This relationship is noted in embryologic growth and development as well as wound healing and has

FIG. 5. Adipocytes and capillaries are not in direct apposition. Transmission electron micrograph of adipose tissue, illustrating a capillary, adipocyte, and surrounding ECM. The adipocyte is in the preadipocyte stage of differentiation, denoted by the numerous lipid pools. The ECM fibers [examples denoted with asterisks (*) in (B) and (C)] possess widths ranging between 30 and 40 nm and interfiber spacing ranging between 50 and 370 nm. (A) 3000 $\times$; (B) 10,000 $\times$, magnified image of (A); (C) 50,000 $\times$, magnified image of (B).

repeatedly been demonstrated experimentally. Our own studies^{17,22,42} and those of others⁴³ demonstrate *de novo* adipogenesis in perivascular tissue around polymer implants. Moreover, the presence of adipose cells follows after the foundation of a vascular network has been established and fat deposition is common after involution of a capillary hemangioma. Microvascular endothelial cells and preadipocytes are not, however, in direct apposition, as they do not possess cognate cell–cell adhesion molecules (Fig. 5). Intercellular signaling instead occurs through secreted or matrix bound factors. A host of such factors from microvascular endothelial cells have been shown to promote preadipocyte proliferation and differentiation during adipogenesis.^{41,44–47} Conversely, established adult adipose tissue is proangiogenic, primarily due to its secretion of growth factors and hormones.^{48–57} Interestingly, despite knowledge of these other factors, including vascular endothelial growth factor, leptin, and adiponectin, the studies in Tables 1–3 only employed FGF-2 to enhance angiogenesis. Adipogenesis is intimately coupled to angiogenesis, and both processes must be considered in development of a clinically translatable strategy for adipose tissue engineering. Complicating the scenario in terms of breast and head and neck reconstruction is the fact that many of the desired implantation areas will be irradiated as part of cancer treatment, severely impeding vascularization and wound healing. This fact will need to be addressed in future adipose tissue engineering strategies.

A final concept that can be gleaned is that an appropriate cell source is required to achieve adipogenesis. That is, a cell source is required for inductive growth of adipose tissue. What is unclear, however, is what the cell source should be. Options investigated to date (see “Seeded cells” column in Tables 1–3) include ADCs, mesenchymal stem cells differentiated along the adipogenic pathway, and the recruitment of resident adipose precursor and stem cells from local tissue depots and/or from vasculature.

CONCLUSION

Tissue engineering burst on to prominence more than 20 years ago, with enticing promises to revolutionize standard reconstructive pathways. While the ability to stimulate cell growth has been thoroughly vetted, unfortunately the limitations of the technology, including tissue permanence, scale-up, and optimization of scaffolds, to name a few, have made modest progress toward the realization of a clinically relevant engineered construct of any tissue type. The first adipose tissue engineering study was published in 1998. Hence, at roughly a decade old, adipose tissue engineering is still in its infancy. Despite the short time frame, a wealth of animal models has been employed to assess adipogenesis *in vivo*. However, we are far from providing a clinically translatable product. This lack of forward progress has encouraged renewed interest in parallel arenas of investi-

gation, including the application of bioprosthesis and composite tissue allotransplantation. The ability to “engineer” a custom three-dimensional living tissue construct to restore a physical deformity without the need for multiple surgical steps and a painful, scarred donor site remains a primary goal of translation scientists and plastic surgeons and their patients. This avenue of study remains the most appealing means to create a composite tissue construct as it avoids the negatives such as incompatibility and immunosuppression inherent to the other technologies. The question then remains the best and most effective means to study tissue engineering toward a clinically relevant goal.

The application of a vascular pedicle and the accordant creation of effective vascular network, coupled with effective scaffold technology, provide key supportive elements, theoretically rendering permanence to the engineered construct. The tissue construct created must demonstrate a complex three-dimensional shape of adequate size to have clinical applicability to current reconstructive problems and demonstrate stability over time. In order to make significant progress over the next decade and to move research into the clinic, it is recommended that (a) a standard rodent model be accepted by the community so that proof-of-principle data among laboratories can be directly shared; (b) standard quantitative assessment techniques be defined to assess structural (e.g., tissue permanence, shape maintenance, and biomechanics) and functional (e.g., vascular density and insulin secretion) aspects of *de novo* adipogenesis; (c) additional studies be conducted in preclinical, longitudinal large animal models; (d) scaffolds be optimized to promote adipogenesis; and (e) attention be placed on deciphering the underlying mechanisms of the interplay between adipogenesis and angiogenesis.

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