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Autologous Fat Grafts: Can We Match the Donor Fat Site and the Host Environment for Better Postoperative Outcomes and Safety?

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Abstract
Purpose of Review Autologous fat grafting is the gold standard for soft tissue repair. The donor fat depot is chosen where a surplus of subcutaneous fat is found. However, the adipose tissues from different parts of the body are not equivalent. Despite the heterogeneity in fat depots, it is still considered that any adipose tissue site is a suitable fat depot donor for transplantation.
Recent Findings Matching embryonic origins and *Hox* code between transplanted stem cells and the host microenvironment emerges as a critical parameter to achieve correct repair in different preclinical models. It has also recently been reported that the individual fat depots routinely used in reconstructive surgery exhibit distinct embryonic origins and express different *HOX* code. An opposite gradient from the upper to the lower body exists between expressions of *HOXC10* and the neural crest marker *PAX3*. This observation raises the question of the choice for the best fat donor site.
Summary Matching between the host tissue and the donor fat sites is a factor that urgently deserves consideration to improve postoperative outcomes and safety of autologous fat grafting.

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Introduction

The use of autologous fat grafting in reconstructive and plastic surgery has been a validated technique for several years. Reconstruction with autologous fat is mainly applied after tumor removal, for breast reconstruction surgery after mastectomy, to repair extensive facial deformities caused by injury, illness, or congenital abnormalities. The treatment's main advantages are that autologous fat is easy to obtain with minimum morbidity for patients. The donor site is chosen where a surplus of subcutaneous fat is found, such as in hips, abdomen, thigh, and the inner sides of the knees. However, the recent scientific literature shows that the individual fat depots are not equivalent. This brings up the question of choosing the most appropriate donor fat site for heterotopic transplantation depending on the grafting recipient site. With some variations, the technique consists in three stages: fat harvesting from donor sites, processing of the aspirate, and reimplantation at the host site [1]. A number of studies have focused on improving the integration of grafted adipose tissue through the investigations of different mechanisms of fat harvesting and processing or the attempts to supplement grafts with other cells or growth factors. Enriching fat grafts with the stromal vascular fraction (SVF) of adipose tissue or with platelet-rich plasma dramatically enhances fat graft survival ([2] and see for recent reviews [3•, 4]). However, there are still controversies and unresolved questions regarding autologous fat grafting due to the unpredictability of postoperative outcomes. The main disadvantages of this technique are variable engraftment and resorption rates, microcalcification, and cyst formations due to fat necrosis.

The differentiation of SVF toward an unwanted cell type after grafting cannot be ruled out. Indeed, clinical results are inconstant, without any clue about the reasons underlying the reconstruction success or failure. Integration of fat graft, its survival, and long-term maintenance are multifactorial. The choice of the donor site is an element to be considered. Autologous fat graft causes the displacement of both mature adipocytes and adipose stem cells form a donor site to a distinct host microenvironment. Indeed, the adipose tissues from different parts of the body are not equivalent. Thus, a better knowledge of the adipose tissue depots used as donor sites and of their interactions with the host environment could be translated towards the clinic.

The question of the most appropriate fat depot as donor site for autologous transplantation emerged recently in the literature. The present review discusses the recent findings that uncovered the importance of a match between the host tissue and the donor fat sites to improve postoperative outcomes and safety of autologous fat grafting.

Adipose Tissues Have Different Properties According to Their Anatomic Locations

Adipose tissues are heterogeneous tissues composed of adipocytes and of a SVF containing different cell types, including the adipose stem cells (Fig. 1). The adipose tissue is now recognized as an endocrine tissue as both adipocytes and SVF secrete numerous cytokines (named adipokines) displaying a variety of biological effects [5]. Importantly, individual fat depots exhibit unique profiles of adipokine and interleukin secretion, including pro-inflammatory cytokines. For instance, a large-scale transcriptomic analysis revealed that *HOX* genes (see below) and inflammatory-related genes are among the genes the most differentially expressed between fat localized in the face and the inner side of knees [6•]. In addition to these differences in the composition of the SVF, two types of adipocytes, i.e., brown and white, having opposite functions coexist in mammals (Fig. 2). White adipose tissue (WAT) is dispersed throughout the body and is mainly involved in energy storage. The two largest depots of white adipose tissues in human are the subcutaneous and the visceral WAT. Visceral and subcutaneous stem cells display distinct intrinsic abilities to proliferate and to undergo differentiation into mature adipocytes [7]. In addition, individual subcutaneous white adipose tissues are not equivalent, in terms of adipose stem cell abundance, proliferation, and differentiation [8]. In contrast to WAT, brown adipose tissue (BAT) is specialized in energy expenditure. Activated BAT consumes metabolic substrate and burns fat to produce heat via the uncoupling protein (UCP)-1 [9]. Brown fat is present in newborns and then disappears from most of the sites but persists in adults in deep organs, i.e., around the kidneys [10] and in the upper part of the body, the neck [11], and face [6•]. A third type of adipocytes mainly recruited in subcutaneous WAT has been identified as brite/beige and correspond to brown-like adipocytes [12–14]. Therefore, the reported heterogeneity in fat depots indicates that the donor adipose tissue sites are not equivalent and suggests that it needs to be chosen according to the site of transplantation.

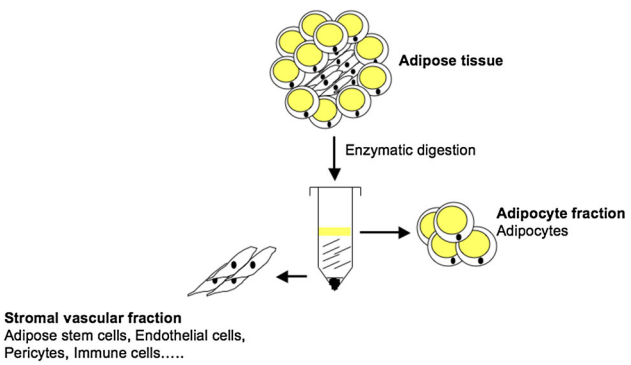

Fig. 1 The adipose tissue is composed of different cell types. Digestion of adipose tissue with collagenase then centrifugation at a low speed separate the tissue in two fractions: the adipocyte fraction containing only adipocytes, and a stromal vascular fraction containing adipose stem cells and different other cell types

Importance to Match the *Hox* Code and the Embryonic Origin Between Transplanted Cells and the Host Environment for Tissue Regeneration in Animal Models

Some publications report that adipocyte viability within different sites is similar suggesting that there is no evidence for a favorable donor site [15, 16]. It has also been reported that some sites may be more suitable than others, based on

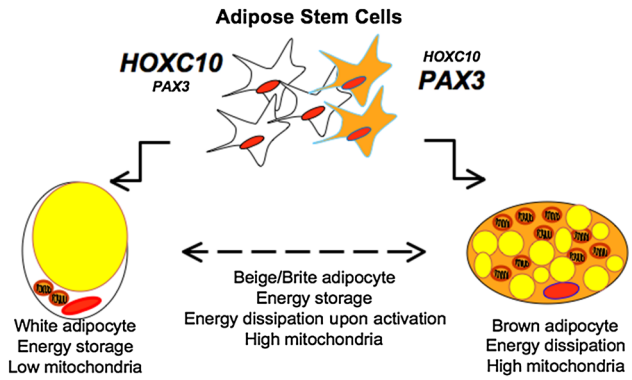

Fig. 2 Different types of adipocytes in Humans. Adipose stem cells generating *white* or *brown* adipocytes have different molecular signature, including different level of *PAX3* and *HOXC10* gene expression. *White* and *brown* adipocytes have different characteristics and functions as indicated. Beige/brite adipocytes are *brown*-like adipocytes dispersed in *white* adipose tissues

the observation that viability of adipocytes is age dependent [17], or based on the amount of adipose stem cells present in distinct sites [18]. However, the fate of adipose stem cells after transplantation has not been taken into account in these studies. Recent observations indicate that the match between the donor site and the host environment is crucial for the behavior of transplanted stem cells. Factors governing the fate of adipose stem cells after transplantation in a heterotopic site remain to be fully identified. The Hox code and the embryonic origin appear to be among them.

Homeobox (*Hox*) genes encode transcription factors determining the positional identity along the anterior–posterior body axis of animal embryos. Recent studies revealed that they also display prominent roles in adult cells. Thirty-eight *HOX* genes were detected in human fat localized in the inner side of knees. Importantly, the *Hox* code, i.e., the *Hox* gene expression profile, was shown to play a critical role in stem cell positional identity. This positional identity is retained after transplantation, and a *Hox* code mismatch between the host environment and grafted stem cells can prevent cells from participating in tissue regeneration. In another study, the authors demonstrated that matching the embryonic origin also plays an unsuspected role in the regeneration processes. They observed that transplantation of tibia-derived *Hox*-positive stem cells originated from mesoderm into the *Hox*-negative environment of the mandible with neural crest origin led to aberrant bone regeneration. In contrast, transplantation of *Hox*-negative neural crest stem cells into a *Hox*-positive mesodermic environment led to a correct repair of the defect [19]. The influence of the *Hox* code has also been highlighted in wound healing [20, 21]. Altogether, these studies demonstrate the plasticity of *Hox*-negative stem cells and their potential to adapt when transplanted in a *Hox*-positive environment. More importantly, the study illustrates that matching the positional identity and the embryonic origins of transplanted cells with that of the host microenvironment appears as a critical parameter to achieve regeneration [22].

Individual Fat Depots Commonly Used in Reconstructive Surgery Exhibit Distinct Embryonic Origins and They Express Specific *HOX* Code

Recent studies revealed that human adipose stem cells display distinct molecular signatures, including the *HOX* code, according to their anatomic location. In addition, lineage tracing approaches in rodents revealed that fat depots have different embryonic origins [23••]. Indeed, in contrast with the previous belief that all adipocytes derive only from mesoderm, adipocytes localized in the face display a neuroectodermal origin whereas adipocytes localized in the other parts of the body originate from mesoderm [24]. As lineage tracing approaches are not feasible in Humans for an obvious reason, molecular studies have been investigated to determine the embryonic origin of human facial and limb fat depots. These studies showed that facial adipose stem cells are *HOX*-negative and of neural crest origin, whereas limb adipose stem cells are *HOX*-positive and likely of mesodermal origin [6•]. Differential properties and *HOX* code between facial and abdominal adipocytes have also been reported [25], in agreement with the conclusions of the lineage tracing studies performed in mice. According to the animal studies showing aberrant repair when tibia stem cells are transplanted in mandible as discussed above, the fate of adipose stem cells localized in inner side of the knee when transplanted in the face may have potential issue that needs to be analyzed. The molecular profile of several fat depots has been reported more recently [26••]. The results, schematized in Fig. 3, showed a gradient of expression of *HOXC10* from the upper to the lower body. An opposite gradient was revealed for expression of *PAX3*, a marker of adipose stem cells of neural crest origin. In addition to the mismatch of the embryonic origin and to the differences of the *HOX* code between these two fat depots, adipose stem cells generate adipocytes presenting a different functional phenotype. In fact, knee and the face fat depots display a white and a brown-like phenotype, respectively [6•]. The consequences of transplanting donor cells raising adipocytes with an opposite metabolic phenotype on the outcome of fat grafting remain to be investigated.

Altogether, these studies highlight that the different fat depots used in clinical practice for plastic and reconstructive surgery have different *HOX* code and embryonic origins. The work further gives a reflection on the request to choose the most appropriate donor site for fat grafting, according to the host environment.

Matching Donor Sites and Mammary Environment for a Better Safety of Post-Mastectomy Breast Reconstruction Surgery

Obesity represents a risk factor for cancer incidence, as it plays a pivotal role providing a permissive tumor microenvironment to initiate and propagate tumor growth. Over-expansion of white adipose tissue increases the risk of developing malignancies through secretion of cytokines by adipose stem cells and adipocytes [27]. Although many recent articles based on clinical series have argued for the safety of autologous fat grafting [28, 29], others based on systematic reviews present more moderate assessments,

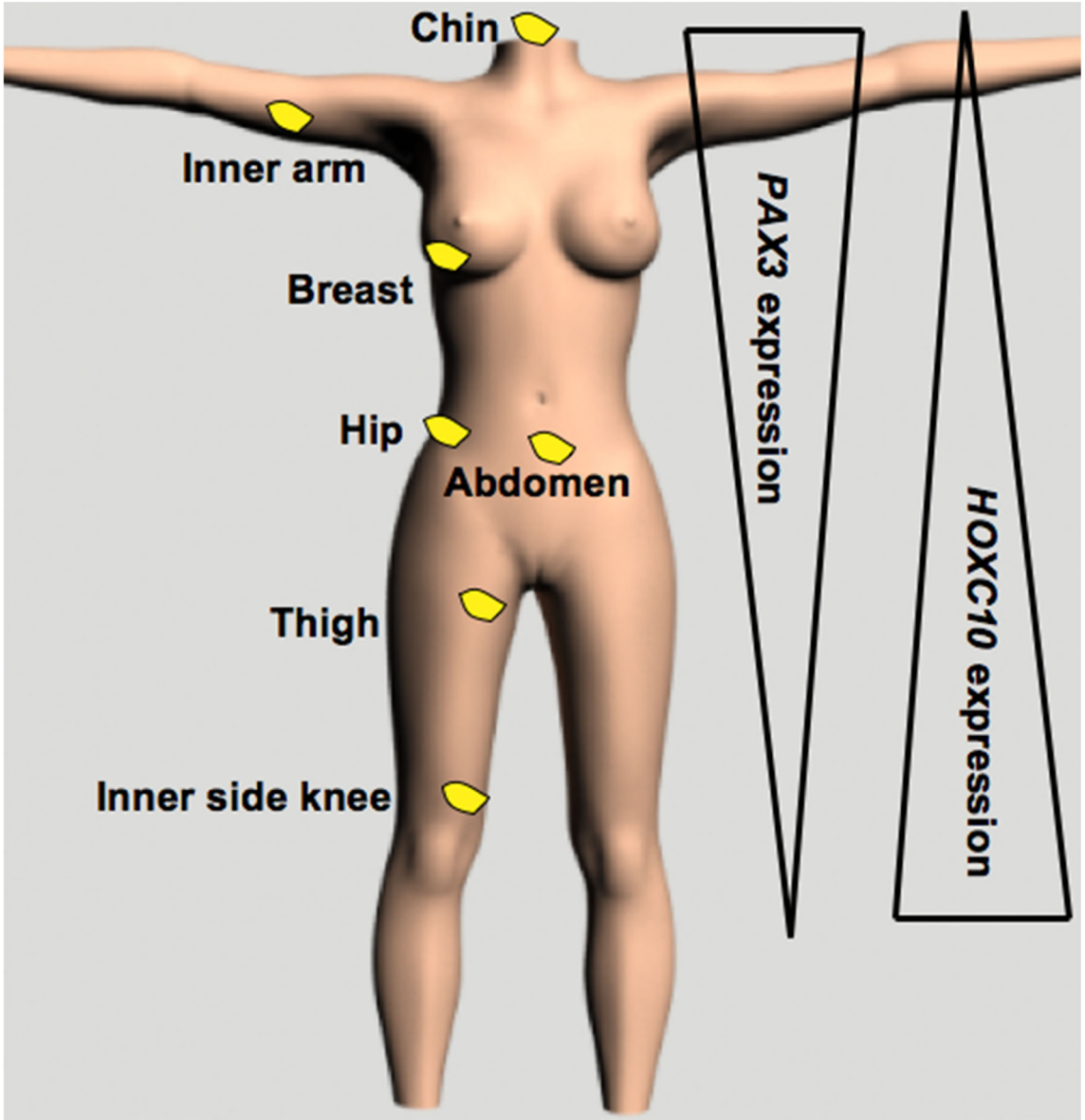

Fig. 3 The fat depots used in reconstructive surgery exhibit different feature. Fat tissue indicated on the scheme has been analyzed for the expression of *PAX3*, a marker on neural crest embryonic origin, and of *HOXC10*. The data revealed an inverse gradient of expression for the two genes

concluding that oncological safety remains unclear and that long-term results and further studies are necessary [30]. An excess of adipose tissue represents a poor prognosis factor for women with a high-grade breast cancer, and adipocytes have been shown to promote both breast cancer cell proliferation and metastatic potential in several murine models [31, 32]. Safety of autologous fat grafting in breast reconstruction surgery after mastectomy remains to be adequately addressed. Traditional breast cancer treatment begins with surgery leading to the excision of the tumor. Then, a radiotherapy and/or chemotherapy are undertaken to remove cancer cells, and finally, autologous fat grafting allows breast reconstruction. There is no clinical evidence of an oncologic risk associated with fat graft for either patients who were previously treated for malignant breast tissue or for subjects embarked on plastic surgery with healthy breast tissue. However, the scientific and the clinical literature present a debate regarding the oncologic risk of the procedure [33, 34••]. It has been shown that a bidirectional cross-talk takes place between mammary adipocytes and cancer breast cells where cancer cells convert white adipocytes into brown adipocytes and induced the release of stored fatty acids, which in turn "feed" cancer cells [35••]. Numerous preclinical studies in animal models indicate that the grafted adipose tissue can stimulate breast cancer cells and promote their metastatic potential [32, 36].

Retrospective clinical data do not eliminate this possibility [34••].

Adipose tissues from different body sites that are used for breast repair may not be equivalent in term of oncogenic risks and selection of the donor site represents a critical factor that deserves to be considered. The work of Foissac and colleagues [26••] proposed a classification of the best molecular match between different donor sites and breast environment. It is now known that cells adjacent to a tumor are not only passive structural elements but are also active actors in tumor progression [35••]. The differential expression of *HOXC10* shown between mammary fat and other fat depots is of particular interest [26••]. *HOXC10* expression is low in breast but higher in other fat depots often used for breast reconstruction, such as in knee and abdomen. Others have shown that *HOXC10* is not only overexpressed in mammary cancer but is involved in the progression of breast cancer [37]. Therefore, two major questions require to be further addressed: what is the impact of transplanting fat depot expressing *HOXC10* in the *HOXC10*-negative mammary environment? Can the molecular mismatch in *HOXC10* aggravate the oncologic risk? The impact of transplantation of fat depots from different localizations on cancer cells deserves further evaluation.

Conclusions

The question regarding the best donor site for grafting into heterotopic sites is a relevant question that merits to be scientifically and clinically investigated. The mechanisms underlying the integration rate of a fat graft are multifactorial. The overview of the most relevant and recent literature indicates that matching the embryonic origin and the *HOX* code between the host and donor sites is a factor to be considered to improve the postoperative outcomes and safety of autologous fat grafting. The impact of mismatch between the fat donor sites and the host environment deserves further evaluation. Development of cellular co-culture models and of preclinical models is required to analyze the interaction between different fat donor sites and the host environment to optimize the clinic practices.

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Compliance with Ethics Guidelines

Conflict of Interest The authors declare no conflicts of interest relevant to this manuscript.

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