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IDEAS AND INNOVATIONS

Reconstructive

Modulation of Cellular Stemness for Enhanced Fat Grafting

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Summary: Low volumetric retention limits the utility of fat grafting. Although inclusion of stem cells and platelet-rich plasma have been proposed to enhance graft retention, accumulating evidence has failed to show a clear benefit. Here, we propose a strategy to pharmacologically enhance stemness of stem and progenitor cell populations in fat grafts to promote increased volume retention and tissue health. We also propose how to integrate stemness-promoting and differentiation-promoting therapies such as platelet-rich plasma, and viability promoting therapies within the common fat grafting workflow to achieve optimal fat grafting results.
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IDEA

Fat grafting can correct volume deficits using autologous adipose tissue. Centrifugation separates lipoaspirate into layers of oil, adipocytes, an infranant layer containing blood, and stromal vascular fraction (SVF) containing nucleated cells (Fig. 1). The SVF contains pericytes, adipocytes, endothelial cells and their progenitors, and mesenchymal stem cells (ASCs). In contrast to progenitor cells that are committed to a defined tissue lineage, ASCs possess versatile differentiation potential, and can differentiate into several lineages including fat, bone, cartilage, muscle, and skin in response to external signals.[1–3] Inclusion of SVF (which contains ASCs) has been used to promote volume retention; however, the benefit remains unclear.[4]

Beyond standardization of graft harvesting, processing, and injection, we propose that application of stem cell modulating adjuncts that (1) promote and maintain stemness, (2) stimulate differentiation, and (3) promote graft viability can promote fat graft volume retention and tissue health. Figure 2 summarizes the proposed interventions within a common fat grafting workflow.

It was recently demonstrated that somatic cells can be chemically reprogrammed into pluripotent stem cells,[5] demonstrating that stemness can be induced and maintained pharmacologically. Chemical reprogramming has also been shown to reverse cellular aging.[6] We propose to apply this phenomenon to promote ASCs in fat grafting.

If effective clinically, pharmacological modulation of stemness of fresh SVF cells with small molecules at point-of-harvest is viable, obviates the need and hassle of genetic interventions, and is highly innovative. Table 1 shows pharmacological agents that promote cellular stemness.[5] Although chemical reprogramming to achieve stemness is a novel phenomenon and, therefore, no clinical evidence exists, we lay out a strategy to accelerate the clinical transition. We propose that intraoperative exposure of donor adipose harvest (ie, in tumescent solution) with stemness-promoting cocktails, as well as co-injecting freshly harvested SVF cells + fat parcels with the stemness-promoting chemical cocktail, enhances graft survival (Fig. 2). Supplementing lipoaspirate with stemness-promoting cocktail may decrease ASC attrition in the immediate term (through block of cellular death and differentiation) and long term (through epigenetic maintenance of stemness pathways). Fat grafting produces an injurious environment, which triggers angiogenesis and also induces adipogenic differentiation, leading to volume retention.[7,8] In the long term, the maintained stem cells can differentiate into adipogenic lineage, while asymmetrically maintaining the naive stem cell population. Preclinical and clinical studies will optimize cocktail components, dosing, delivery formulation (ie, liposomal), and length of pretreatment.

We propose the following concerns regarding the use of differentiation promoting adjuncts, such as platelet-rich plasma (PRP) in the absence of stem cell promoting therapy. Differentiating promoting adjuncts (1) tend to induce transitory effects because terminally differentiated

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adipocytes have no ability to regenerate; (2) if repeated, will theoretically contribute to the attrition of ASCs; and (3) may be ineffective in the absence of stem or progenitor cells. Therefore, we propose that promoting the stemness and survival of ASCs during grafting will increase efficacy of PRP therapies.

We propose preoperative and intraoperative local administration of pro-angiogenic agents and cytoprotective antioxidants, and metabolo-protective agents will enhance graft take, mitigate ischemia, and reduce ASC attrition (Table 1 and Fig. 2).

Takeaways

Question: How to enhance long-term volume retention in fat grafting?

Findings: We propose that beyond including stem cells and growth factors in fat grafts, enhancing stemness of stem cells in fat grafts pharmacologically may provide long-term benefits in volume retention. We identified pharmacologic agents that enhance cellular stemness and developed therapeutic approaches to adapt this to fat grafting.

Meaning: Incorporating stem cell enhancing agents during fat grafting may enhance long-term volume retention.

DISCUSSION

Boosting stemness enhances graft acceptance, counters cell attrition, prevents long-term volume decline, and provides a lasting advantage beyond simple ASC graft inclusion.

Stemness, an intrinsic cellular property, is orchestrated by intricate epigenetic modulation and crucial transcription factors such as octamer-binding transcription factor 4, SRY-box transcription factor 2, NANOG, and MYC. This characteristic dissipates as cells undergo the process of differentiation. Injection of cells that have been reprogrammed to pluripotent stem cells in vitro is clinically impractical.[5] Future directions should investigate the effects of stemness-promoting factors on short-term and long-term increase in fat volume.

In the microenvironment, growth factors and cytokines propel stem cell differentiation and boost mature cell functionality. Insulin, corticosteroids, Wnt ligands, BMPs, and adipokines like adiponectin stimulate adipocyte differentiation, offsetting initial adipocyte decline due to apoptosis. Vascular endothelial growth factor (VEGF) enhances endothelial progenitor cell shift to endothelial cells, aiding microvascular network development. PRP,

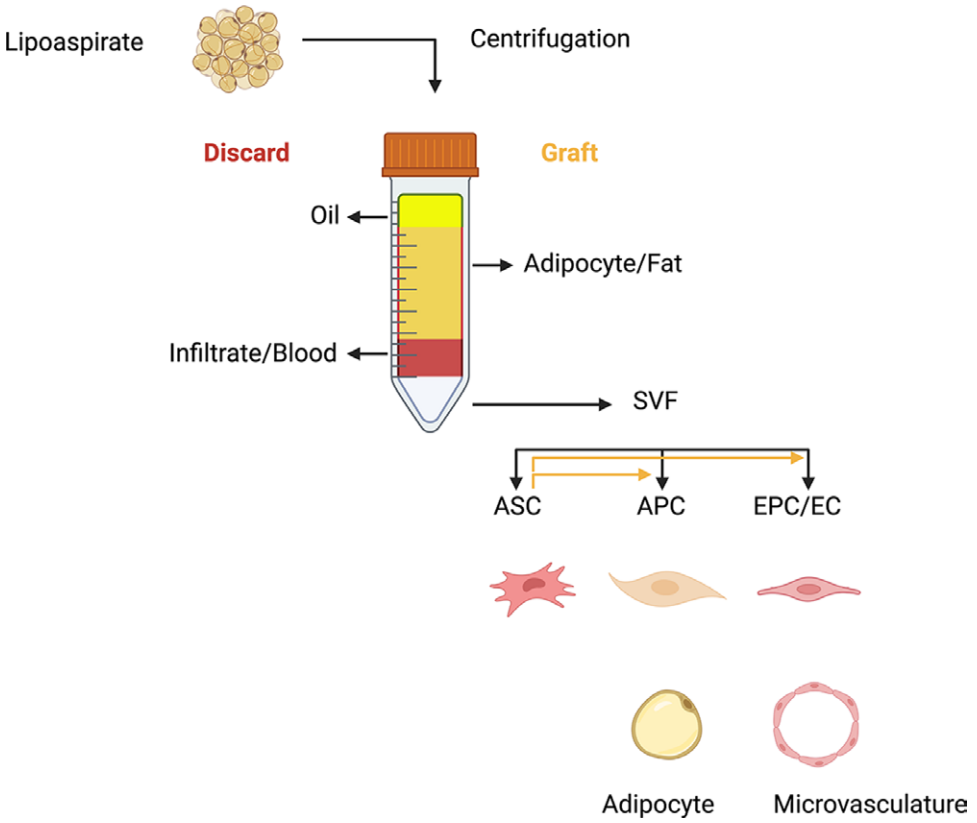

Fig. 1. Common workflow for processing lipoaspirate. The SVF contains nucleated cells, and can be combined with the fat fraction to promote volume retention. ASCs have the ability to differentiate into several lineages, including adipocytes, which may theoretically counter volumetric decline, and endothelial cells, which may increase graft take through angiogenesis. APC, adipocyte progenitor cell; EPC, endothelial progenitor cell; EC, endothelial cell. Created with BioRender.com.

rich in growth factors, is used to enhance fat grafting outcomes, yet conclusive benefits remain uncertain.[9]

Similarly, new proposed therapies to combine ASCs with growth factor RNAs and/or exosomes will likely only lead to transitory effects due to limited half-lives and failure to induce stemness. Including growth factors, either as RNAs or exosomes containing growth factors and/or growth factor RNAs, similar to PRP, will tend to promote cellular differentiation.[8,10] There would be limited benefit in ASC-poor grafts. ASC-rich grafts would see a short-term increase in volume, but the benefit would decrease in the long-term due to loss of ASCs through repetitive differentiation. Moreover, RNAs and exosomes are subject to a more stringent regulatory barrier due to their biological nature.

Table 1. Pharmacologic Agents that May Promote Stemness, Differentiation, and Viability of Fat Grafts

	Pharmacologic Agents
Stemness promoting	C6NYSA (CHIR99021, 616452, TTNPB, Y27632, SAG, ABT869), tranylcypromine, PDGF-BB, 5-AZA
Differentiation promoting	–PRP –Adipogenic: insulin, corticosteroids, TGF-beta, BMPs, Wnts (Wnt 10b), adipokines. –Vasculogenic: VEGF, BMP4, ETV2, Nrp1, ephrinB2
Viability promoting	Nitric oxide–enhancing agents, arginine, B vitamins (particularly B1 and B3), vitamin E, vitamin C, glutathione/antioxidants, N-acetylcysteine

5-AZA, 5-azacytidine; BMPs, bone morphogenetic proteins; nrp1, neuropilin 1; PDGF-BB, Platelet derived growth factor (beta, beta); SAG, smoothened agonist; TGF, transforming growth factor.

The enduring efficacy of fat grafts hinges primarily upon vascularization. Initial nutritional supply within the first 48 hours relies on plasmatic imbibition, followed by capillary ingrowth during the inosculatory phase. Ischemia chiefly contributes to the immediate attrition of ASCs during fat grafting, a situation that can worsen in inadequately vascularized recipient sites. Hence, we posit that adjuncts fostering viability and expediting vascular integration into grafts will (1) augment graft survival, (2) enhance graft metabolism, and (3) mitigate ischemia throughout both early and late stages.

Adipose graft viability relies on the emergence of microvascular proliferation and neoangiogenesis that interconnect with the vascular networks of the recipient site (hybrid vascularization). Various vitamins and nitric oxide-promoting agents boost angiogenesis, simultaneously delivering nutritional and metabolic sustenance that enhances graft success. Additionally, antioxidants can foster microvascular growth,

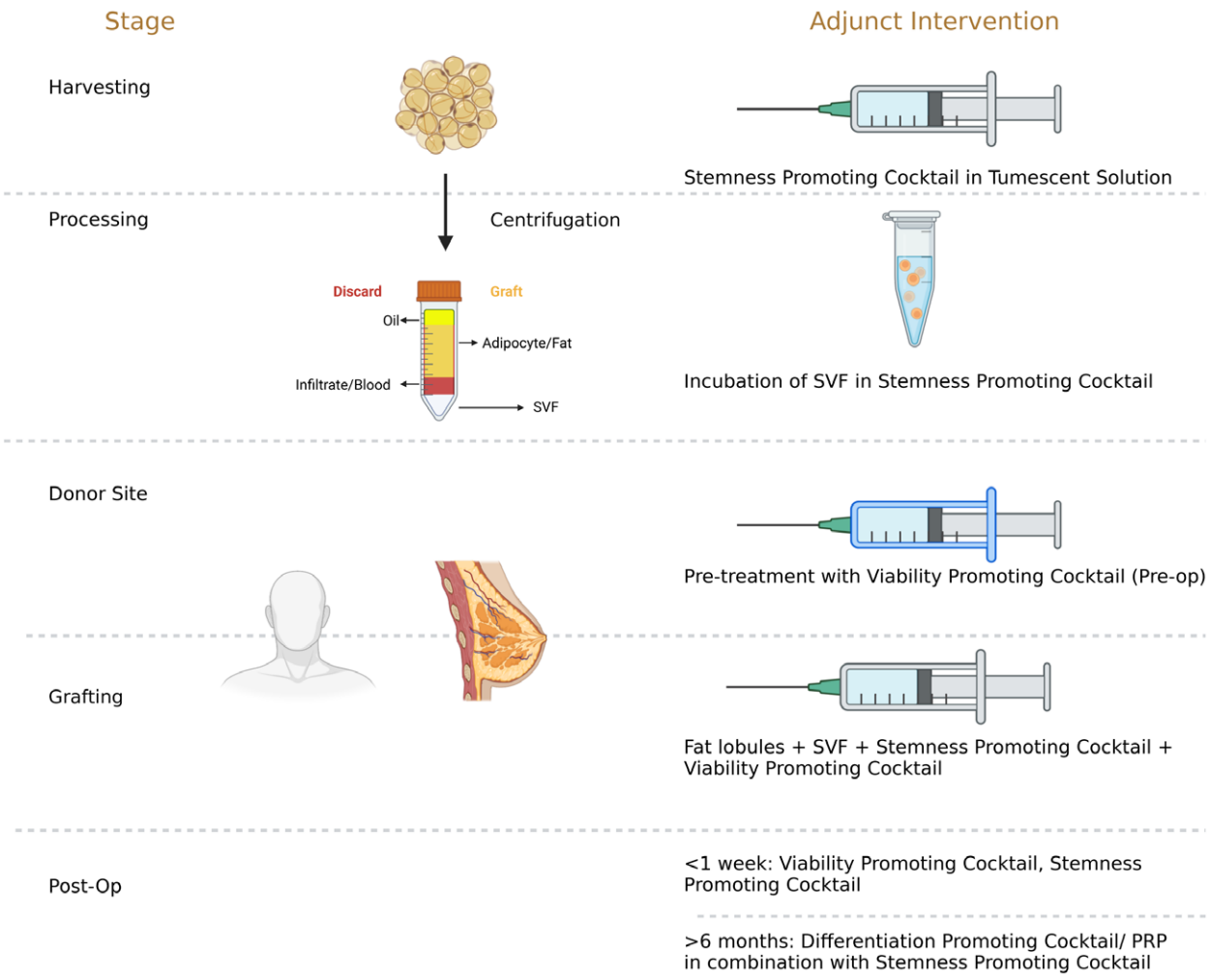

Fig. 2. Proposed intervention and fat grafting workflow. Created with BioRender.com.

diminishing ischemia-reperfusion injury's impact on ASCs during both early and late grafting stages,[8] thereby alleviating another source of cytotoxicity. This approach could forge innovative pathways for enhancing fat grafting and sustaining volume. Analogous techniques could also be extended to bolster the viability of other grafts and flaps, especially in cases complicated by microvascular disease and other risk factors.

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DISCLOSURES

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