Title: USE OF LIPOIC ACID IN CELL TRANSPLANTATION

Abstract: The present invention provides lipoic acid or derivatives thereof for use in graft stabilization of fat grafts. Mixing of lipoic acid or a derivative thereof with adipocytes or adipose tissue to be transplanted into a subject leads to more successful fat transplantation in soft tissue augmentation or reconstruction. Such methods may also be used in the transplantation of adult stem cells or other cells derived from fat tissue. Other agents such as antioxidants, vitamins, pharmaceutical agents, or osmotic protectants may also be added to the lipoic acid/cell compositions for cell transplantation.
USE OF LIPOIC ACID IN CELL TRANSPLANTATION

Related Applications


Background of the Invention

[0002] Soft tissue injuries and malformations secondary to trauma, congenital defects, infections, and oncologic resections are a source of significant morbidity in patients. At present autologous free flap reconstruction or local advancement flaps are the workhorses of reconstructive modalities for significant soft tissue and bony defects. While pedicled flaps and free flap reconstructions offer powerful tools for reconstruction, they are not without potentially serious side effects and donor site morbidity.

[0003] Autologous fat transplantation has been used in soft tissue reconstruction but is unpredictable. The advantage of using lipo-aspirated fat is two-fold: 1) minimal donor site morbidity providing a safe and readily accessible source for autologous cells, and 2) these procedures can be performed relatively easily without the concern for ischemic complications and early graft failures associated with vascularized free flaps. However, to date free fat grafts have been plagued with unpredictable high levels of reabsorption and resultant irregularities. Free fat graft failures and volume reduction appear to be related to mechanical stresses resulting in membrane damage from harvesting, early ischemic changes, and nutrient deprivation and insufficient vascular supply to the graft. These stressors lead to apoptosis and cell death. Subsequent graft reabsorption results from removal of dead cellular debris following revascularization. This leads to inconsistent and undesirable results for soft tissue restoration. Since fat transplantation was first described by Neuber in 1893, little has been achieved to improve the results of free fat grafts. Thus far, efforts to attenuate the initial ischemic insult to cells until sufficient vascularity can be established have been met with only modest results. Thus, improving the vascular supply of the fat transplant alone may not be sufficient to greatly improve the results of fat transplantation. Preventing damage to cells during the procurement, handling/storage, and/or transplantation of the fat graft is also important.
There remains a need for more successful transplantation of adipose tissue or cells derived from adipose tissue (e.g., adipocytes, stem cells) in cosmetic and reconstructive surgery. The ability to transfer a large volume of autologous adipose tissue for soft tissue reconstruction would provide a novel reconstructive option for potentially millions of patients, without the associated donor site morbidities. Additionally, it would provide a powerful tool for patients who have poor donor site options, and patients with the inability to tolerate the extended operating times required in flap reconstructions.

Summary of the Invention

The present invention stems from the recognition that damaged cells in fat grafts become apoptotic and eventually are resorbed by the recipient's body. Preventing damage to the cells, such as damage during procurement of the graft, handling of the graft, processing of the graft, and/or the transplantation procedure, in such grafts may allow for more successful and predictable fat transplantation.

Surprisingly, it was discovered that lipoic acid and derivatives thereof are useful in improving the success of fat transplantation. Lipoic acid and derivatives thereof have been found to provide strong anti-oxidant effects thereby reducing inflammation and oxidative stress and/or to provide stabilizing effects to mitochondrial function, glucose transport, and/or insulin sensitivity. It was found that when lipoic acid or derivatives thereof were added to the cells of the graft (e.g., cells in fat transplants), the lipoic acid allowed for graft stabilization, and the lipoic acid treatment significantly reduced long-term reabsorption of the graft.

As used herein, the term "lipoic acid" refers to thioctic acid or thioctinic acid (1,2-dithiolane-3-pentanoic acid; 1,2-dithiolane-3-valeric acid), \( \text{C}_8\text{H}_{14}\text{O}_2\text{S}_2 \), formula weight 206.32, and derivatives thereof. In certain embodiments, "lipoic acid" has the following formula which is the oxidized form:

\[
\text{H}_2\text{C}_6\text{O}_4\text{S}_2\text{O}_2\text{H}
\]

In other embodiments, the reduced form of lipoic acid is used (shown below).

\[
\text{H}_2\text{S}_2\text{C}_6\text{O}_4\text{H}
\]
In still other embodiments, a mixture of the oxidized and reduced forms of lipoic acid is used to stabilize cells and improve cell transplantation.

[0008] The present invention provides methods wherein the cells or tissue to be transplanted are mixed with lipoic acid or derivatives thereof at the appropriate concentration and are then transplanted into the recipient (*e.g.*, a human) at the desired transplant site (*e.g.*, face, lips, breast). Any cells or tissue may be transplanted using the inventive technology. In certain embodiments, the tissue is adipose tissue. In certain embodiments, the cells are derived from adipose tissue. In certain embodiments, the cells are part of a fat graft (*i.e.*, adipose tissue) that contains different types of cells including, but not limited to, adipocytes, stromal cells, epithelial cells, endothelial cells, fibroblasts, and blood cells. In certain embodiments, the cells are adipocytes. In certain embodiments, the cells are fibroblasts. In certain embodiments, the cells are stem cells. In certain embodiments, the cells are stromal cells. In certain embodiments, the cells are endothelial cells. The cells may be mixed with lipoic acid or a derivative thereof at the time of harvesting or at the time of processing or storage, or the cells may be mixed with lipoic acid or derivatives thereof just prior to transplantation. In certain embodiments, the site where the adipose tissue is to be removed is injected with a composition including lipoic acid before the tissue is removed from the donor. In other embodiments, lipoic acid or derivative thereof is added to the cells after they have been removed from the donor. Lipoic acid or a derivative thereof may also be added to the cells at the time of processing or storage, or the cells may be mixed with lipoic acid or a derivative thereof just prior to transplantation. The cells or tissue may be washed or otherwise processed with a composition that includes lipoic acid or a derivative thereof. In certain embodiments, the lipoic acid or a derivative thereof is washed from the graft prior to implantation.

[0009] The cell/lipoic acid composition may also include other agents. For example, the composition may include agents that further protect or stabilize the cells to be transplanted. In certain embodiments, the composition includes vitamins, minerals, additional antioxidants, osmotic protectants, viscosity enhancers, coenzymes, membrane stabilizers, lipids, carbohydrates, hormones, growth factors, anti-inflammatory agents, polynucleotides, proteins, peptides, alcohols, organic acids, and/or small organic molecules.

[0010] The present invention provides compositions of adipocytes and lipoic acid or derivatives thereof for use in fat transplantation. In certain embodiments, the present invention provides compositions of stem cells and lipoic acid. The stem cells may be adult stem cells derived from fat tissue or the stem cells may be obtained from other sources. Other cells derived from fat tissue (*e.g.*, stem cells, fibroblasts, stromal cells) may also be used in the
invention. The inventive cell/lipoic acid compositions may include other agents e.g.,
antioxidants, vitamins, lipids, proteins, peptides nucleic acids, hormones, growth factors,
carbohydrates, and pharmaceutical agents.

[0011] In another aspect, the invention provides kits useful in transplanting fat using the
inventive compositions and methods. The kits may include all or a subset of all the
components necessary for transplanting adipose tissue or fat-derived cells into a subject. The
kits may include, for example, lipoic acid, buffered solution for washing cells, device for
washing cells, cells, syringe, needle, alcohol swabs, anesthetics, antibiotics, antioxidants,
vitamins, lipids, carbohydrates, hormones, growth factors, etc. In certain embodiments, a
container or syringe used in harvesting the cells may have in it lipoic acid so that the harvested
cells are immediately contacted with lipoic acid or derivatives thereof. In certain embodiments,
the cells are acquired from the patient to receive the cells (i.e., an autologous graft). In certain
embodiments, the components of the kit are steriley packaged for convenient use by the
surgeon or other health care professional. The kit may also include instructions for using lipoic
acid or derivatives thereof and optionally other agents in the harvesting/transplantation
procedure. The kit may provide the necessary components for a single use. The kit may also
include packaging and information as required by a governmental regulatory agency that
regulates pharmaceuticals and/or medical devices.

Brief Description of the Drawing

[0012] Figure 1. Bar graph showing reabsorption by weight of treatment groups at six
weeks (compared to dehydrated ten-day weight). Lipoic acid (LA) (black bar) demonstrated
statistically significant differences in reabsorption at six-weeks when compared to saline
controls (grey bar).

[0013] Figure 2. Bar graph showing apoptosis (RFUs) as a function of explantation time
during the first ten-days of engraftment. Significant differences are noted in LA treated groups
(black bars) when compared to saline controls (grey bars) at days 7 and 11.

[0014] Figure 3. Bar graph showing raw luminescence (raw RLU), representing ATP
activity, at six-weeks post-treatment and implantation. LA (black bar) demonstrates higher
levels of ATP activity when compared to saline (grey bar) treated fat.

[0015] Figure 4. Bar graph showing DNA content for sampled lobules at six weeks. LA
(black bar) demonstrated higher levels of DNA content at six weeks compared to saline (grey
bar).
Figure 5. Photographs showing histology of samples post-explantation at six-weeks stained with H&E. Saline samples (left) demonstrated architectural disruption, fibrosis, and oil cysts. By comparison the LA treated samples (right) demonstrated relatively normal adipose architecture and an absence of fibrotic reactions was notable.

Definitions

"Adipose tissue," as used herein, refers to tissue that is composed of adipocytes. Adipose tissue is typically loose connective tissue composed of adipocytes. The main role in the body is to store energy in the form of fat; however, adipose tissue also insulates and protects the body as well as acting as an endocrine organ. Adipose tissue can be white adipose tissue or brown adipose tissue. The term "adipose tissue" is used interchangeably with "fat tissue."

"Anti-inflammatory agent," as used herein, refers to any substance that inhibits one or more signs or symptoms of inflammation.

The term "approximately" in reference to a number generally includes numbers that fall within a range of 5% in either direction of the number (greater than or less than the number) unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

The terms "polynucleotide", "nucleic acid", or "oligonucleotide" refer to a polymer of nucleotides. The terms "polynucleotide", "nucleic acid", and "oligonucleotide", may be used interchangeably. Typically, a polynucleotide comprises at least two nucleotides. DNAs and RNAs are polynucleotides. The nucleotide polymer may include natural nucleosides (i.e., adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxycytosine, deoxyguanosine, and deoxythymidine), nucleoside analogs (e.g., 2-amino-adenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, C5-propynylcytidine, C5-propynyluridine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-methylcytidine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, 0(6)-methylguanine, and 2-thiocytidine), chemically modified bases, biologically modified bases (e.g., methylated bases), intercalated bases, modified sugars (e.g., 2'-fluororibose, 2'-methoxyribose, 2'-aminoribose, ribose, 2'-deoxyribose, arabinose, and hexose), or modified phosphate groups (e.g., phosphorothioates and 5'-N phosphoramidite linkages). Enantiomers of natural or modified nucleosides may also be used. Nucleic acids also include nucleic acid-based therapeutic agents, for example, nucleic acid ligands, siRNA, short hairpin RNA, antisense oligonucleotides, ribozymes, aptamers, and SPIEGELMERS™, oligonucleotide ligands

[0021] A "polypeptide", "peptide", or "protein" comprises a string of at least three amino acids linked together by peptide bonds. The terms "polypeptide", "peptide", and "protein", may be used interchangeably. Peptide may refer to an individual peptide or a collection of peptides. Inventive peptides preferably contain only natural amino acids, although non natural amino acids (i.e, compounds that do not occur in nature but that can be incorporated into a polypeptide chain) and/or amino acid analogs as are known in the art may alternatively be employed. Also, one or more of the amino acids in a peptide may be modified, for example, by the addition of a chemical entity such as a carbohydrate group, a phosphate group, a farnesyl group, an isofarnesyl group, a fatty acid group, a linker for conjugation, functionalization, or other modification, etc. In one embodiment, the modifications of the peptide lead to a more stable peptide (e.g., greater half-life in vivo). These modifications may include cyclization of the peptide, the incorporation of D-amino acids, etc. None of the modifications should substantially interfere with the desired biological activity of the peptide.

[0022] The terms "polysaccharide" and "carbohydrate" may be used interchangeably. Most carbohydrates are aldehydes or ketones with many hydroxyl groups, usually one on each carbon atom of the molecule. Carbohydrates generally have the molecular formula C_{n}H_{2n}O_{n}. A carbohydrate may be a monosaccharide, a disaccharide, trisaccharide, oligosaccharide, or polysaccharide. The most basic carbohydrate is a monosaccharide, such as glucose, sucrose, galactose, mannose, ribose, arabinose, xylose, and fructose. Disaccharides are two joined monosaccharides. Exemplary disaccharides include sucrose, maltose, cellobiose, and lactose. Typically, an oligosaccharide includes between three and six monosaccharide units (e.g., raffinose, stachyose), and polysaccharides include six or more monosaccharide units. Exemplary polysaccharides include starch, glycogen, and cellulose. Carbohydrates may contain modified saccharide units such as 2'-deoxyribose wherein a hydroxyl group is removed, 2'-fluororibose wherein a hydroxyl group is replace with a fluorine, or N-acetylglucosamine, a nitrogen-containing form of glucose, (e.g., 2'-fluororibose, deoxyribose, and hexose). Carbohydrates may exist in many different forms, for example, conformers, cyclic forms, acyclic forms, stereoisomers, tautomers, anomers, and isomers.

[0023] "Small molecule" refers to organic compounds, whether naturally-occurring or artificially created (e.g., via chemical synthesis) that have relatively low molecular weight and that are not proteins, polypeptides, or nucleic acids. Small molecules are typically not polymers with repeating units. In certain embodiments, a small molecule has a molecular...
weight of less than about 1500 g/mol. Also, small molecules typically have multiple carbon-carbon bonds and may have multiple stereocenters and functional groups.

"Subject," as used herein, refers to an individual to whom an agent is to be delivered, e.g., for experimental, diagnostic, and/or therapeutic purposes. Preferred subjects are mammals, particularly domesticated mammals (e.g., dogs, cats, etc.), primates, or humans. In certain embodiments, the subject is a human. In certain embodiments, the subject is an experimental animal such as a mouse or rat. A subject under the care of a physician or other health care provider may be referred to as a "patient."

"Pharmaceutical agent," also referred to as a "drug," is used herein to refer to an agent that is administered to a subject to treat a disease, disorder, or other clinically recognized condition that is harmful to the subject, or for prophylactic purposes, and has a clinically significant effect on the body to treat or prevent the disease, disorder, or condition. Therapeutic agents include, without limitation, agents listed in the United States Pharmacopeia (USP), Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th Ed., McGraw Hill, 2001; Katzung, B. (ed.) Basic and Clinical Pharmacology, McGraw-Hill/Appleton & Lange; 8th edition (September 21, 2000); Physician's Desk Reference (Thomson Publishing), and/or The Merck Manual of Diagnosis and Therapy, 17th ed. (1999), or the 18th ed (2006) following its publication, Mark H. Beers and Robert Berkow (eds.), Merck Publishing Group, or, in the case of animals, The Merck Veterinary Manual, 9th ed., Kahn, C.A. (ed.), Merck Publishing Group, 2005.

**Detailed Description of Certain Embodiments of the Invention**

The present invention stems, at least in part, from the recognition that the transplantation of cells (e.g. adipocytes or adipose tissue) in cosmetic and reconstructive surgery may be improved by the addition of lipoic acid or derivatives thereof to the cells in the graft.

Aspects of the invention are based on the finding that fat grafts undergo ischemic injury during the liposuction harvesting process, followed by a prolonged period of ischemia and subsequent reperfusion during engraftment. Untreated fat undergoes apoptosis and programmed cell-death during this period of acute injury, leading ultimately to high levels of graft reabsorption.

Lipoic acid is an organo-sulfur molecule with a carboxylic acid group and an aliphatic chain. It is a known component of aerobic cellular respiration, acting as a cofactor within the pyruvate dehydrogenase complex. Along with acetyl-CoA it reduces NAD+ to
NADH in the citric acid cycle. Lipoic acid has strong antioxidant properties and has been shown in animal models to modulate oxidative stress (Packer 1998; Roy and Packer 1998). In ischemia-reperfusion studies lipoic acid has been shown to reduce apoptosis (Diesel, Kulhanek-Heinze et al. 2007) and cell death in a rat liver model (Bustamante, Lodge et al. 1998; Muller, Dunschede et al. 2003; Duenschede, Erbes et al. 2007). Using a rat liver model authors have shown a dramatic reduction in the inflammatory cytokine storm associated with severe portal ischemia. In these studies reductions in monocyte activation, myleperoxidase levels, caspase activity, and dramatic improvements in liver histology, compared to control animals were shown (Dulundu, Ozel et al. 2007).

Administration of lipoic acid has been shown to reduce lipopolysaccharide (LPS) related inflammatory responses by activating the phosphoinositide 3-kinase/Akt signaling pathway (in a rat sepsis model) (Muller, Dunschede et al. 2003). This activation results in a strong reduction of NF-κB related activity (CAMs upreglation, TNFa, MCP-1 activation) (Bustamante, Lodge et al. 1998; Kiemer, Muller et al. 2002). Modulation of the PI 3-kinase/Akt pathway along with direct scavenging of free radicals, elaborated during ischemia, and reperfusion, result in dramatic end-organ protection in rat models.

Apart from lipoic acid’s effects on the inflammatory cascade and reduction of ischemia-reperfusion injury, it also has strong adipocyte specific signaling effects. Lipoic acid has been shown to improve adipocyte mitochondrial function, insulin sensitivity, and glucose uptake (Rudich, Tirosh et al. 1999; Moini, Tirosh et al. 2002; Cho, Moini et al. 2003). The R-alpha-lipoic acid enantiomer has been shown in vitro to stimulate adipocyte glucose uptake; this action appears to be related to insulin receptor sensitivity (Cho, Moini et al. 2003). Other studies have subsequently demonstrated not only an increase in intracellular glucose transport, but also, an increase in PPAR-gamma expression, and mitochondrial transcription factor A (Tfam) expression (Shen, Liu et al. 2008). Furthermore, recent studies have shown that adipocytes under oxidative stress develop significant insulin resistance (Rudich, Tirosh et al. 1999). This resistance appears to be ameliorated by lipoic acid mediated expression of GLUT4, leading to increased glucose uptake (Pessler, Rudich et al. 2001).

Aspects of the invention are based on the recognition of the similarities between the rat liver model of injury and fat engraftment in the mouse, and the inventive choice of lipoic acid for fat graft protection. It was recognized that lipoic acid might be a protective agent for fat undergoing oxidative (ischemic) stress by supporting intra-cellular metabolism. It was found that lipoic acid can reduce the oxidative stressors on ischemic grafts during the early
engraftment period and that addition of lipoic acid to cells results in improved long-term fat-graft survival.

[0032] While not wishing to be bound by any particular theory, it is thought that lipoic acid modulates two significant pathways for cell death in adipocytes. Studies have shown in vitro that adipocytes and adipose derived MSCs undergo oxidative stress and mitochondrial dysfunction, while ischemic and nutrient deprived, leading to apoptosis (Zhu, Chen et al. 2006; Potier, Ferreira et al. 2007). Fat grafts at implantation are ischemic, nutrient deprived, and undergo subsequent reperfusion injury. The combination of both ischemia and nutrient depletion, leads to massive mitochondrial dysfunction, impaired glucose transport, and inflammatory activation. In certain embodiments, lipoic acid provides a strong anti-oxidant effect extra-cellularly, e.g., reducing inflammation and oxidative stress. Additionally, in certain embodiments, lipoic acid through multiple pathways intracellularly stabilizes e.g., mitochondrial function, glucose transport, and insulin sensitivity.

[0033] Aspects of the invention are based at least in part on the recognition that the combination of these effects, i.e intra- and extra-cellular effects of lipoic acid, when lipoic acid is added to the cells in the graft, allows for graft stabilization e.g., while a neovascular network is established. In certain embodiments, lipoic acid treatment significantly reduces long-term reabsorption in free fat grafting. Preventing injury to the cells reduces the extent of apoptosis and cell death in the graft and aids in improving the success and consistency of fat transplants in soft tissue restoration, reconstruction, or augmentation. The present invention provides lipoic acids, compositions, and methods for improving fat transplantation in a subject (e.g., humans).

Lipoic acid

[0034] As used herein, the term "lipoic acid" refers to thiocetic acid or thioctic acid (1,2-dithiolane-3-pentanoic acid; 1,2-dithiolane-3-valeric acid), C₈H₁₄O₂S₂, formula weight 206.32, and derivatives thereof, such as its reduced form dihydrolipoic acid, a lipoic or dihydrolipoic acid ester, a lipoic or dihydrolipoic acid amide, a lipoic or dihydrolipoic acid salt (hereinafter referred to collectively as lipoic acid or LA for ease of reference). In certain embodiments, "lipoic acid" may have the following formula (oxidized form):

\[ \text{Lipoic acid} \]
In other embodiments, "lipoic acid" may be in the reduced form:

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HS  \[O\]
SH
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"Lipoic acid" as used herein includes the isolated R-enantiomer, isolated S-enantiomer, and racemic mixtures of both enantiomers (racemate). Various forms of lipoic acid are described for example in U.S. Application No. 2004/0265345; U.S. Pat. Nos. 5,569,670; 5,693,664; 5,728,735; 5,621,117; 5,650,428, each of which is incorporated herein by reference in their entirety.

[0035] "Lipoic acid derivatives" include, but are not limited to, thiocctic acid esters, particularly C₂ to C₂₄ alkyl esters such as fatty acid esters, especially C₄ to C₁₈ fatty acid esters commercially available or prepared from common oil feedstocks used dermatologically, such as coconut and/or palm oil; amides, particularly an oxidized form sold commercially (Ryan Scientific, Mt. Pleasant, SC) DL-lipoamide or DL-6,8-thioctic acid amide (C₈H₁₅NOS₂), and amides isolated from or mimicking naturally occurring lipoamides, and salts, particularly alkali metal salts, anhydrides and specifically the reduced form, dihydrolipoic acid, and its esters, amides and salts, dihydrolipoic acid esters (particularly C₄ to C₁₈ fatty acid esters) and dihydrolipoamides such as the reduced form of DL-lipoamide and dihydrolipoamides derived from or mimicking naturally occurring lipoamides, and dihydrolipoic acid N,N-dimethyl,N-2-amidoethyl lipoate (LA-Plus) described by Sen, C. K., et al. (Free Radical Biol. Med., 1998, 25: 89), which exhibits increased cellular uptake and biological activity. Lipoic acid derivatives and sythesis are described, for example, in U.S. Patent Nos: 6,605,637; 6,331,559; 6,353,011; 6,090,842; 5,489,694, each of which is incorporated herein by reference in their entirety.

[0036] Derivatives may also include those involving other reactive groups known to those skilled in the art. As used herein, the term "derivatives" includes metabolic precursors of lipoic acid as well as metabolites and degradants of lipoic acid. In certain embodiments, wherein lipoic acid derivatives are employed they should be functionally equivalent to lipoic acid, that is they should be at least 50% equivalent, or at least 60%, 75%, 80%, 90%, 95%, 99%, 100% equivalent to the function of lipoic acid.

[0037] Lipoic acid is both fat- and water-soluble and, in certain embodiments, can be used in either lipid- or aqueous-based compositions. In certain embodiments, lipoic acid readily crosses cellular membranes and disperses in extra-cellular and intra-cellular tissue compartments, cells and/or cell suspensions in vivo and in vitro.
[0038] Lipoic acid was originally identified as a bacterial growth factor present in the water-soluble fraction of liver and yeast. It was found to be necessary for the oxidative decarboxylation of pyruvic acid by *Streptococcus fecalis* and for the growth of *Tetrahymena gelii*, and replaced acetate for the growth of *Lactobacillus casei*. It has been variously known as acetate replacing factor, protogen A, and pyruvate oxidation factor.

[0039] While not wishing to be bound to any theory, it is thought that lipoic acid acts as a free radical scavenger and neutralizer, and prevents, *e.g.*, the cross-linking of cell membranes. Lipoic acid modulation of free radicals and other oxidative species also affects gene expression, including expression of nuclear factor kappa-B (NF-κB), nitric oxide synthetase, and other mediators at various stages of proinflammation and inflammation.

[0040] The present invention is based, at least in part, on the discovery that lipoic acids which provides strong anti-oxidant effects thereby reducing inflammation and oxidative stress and/or further provides stabilizing effects to mitochondrial function, glucose transport, and/or insulin sensitivity are useful in improving the success of fat transplantation. In certain embodiments, lipoic acid or derivatives thereof when added to the cells in the graft (*e.g.*, cells in fat transplants or cells derived from fat tissue, *e.g.*, stem cells, fibroblasts, epithelial cells, stromal cells) allows for graft stabilization while a neovascular network is established. In certain embodiments, lipoic acid treatment significantly reduces long-term reabsorption in free fat grafting. Such lipoic acids may be used in conjunction with other methods of improving the success of fat transplantation including, for example, improving the vascular supply or adding other agents (*e.g.*, membrane stabilizer, antioxidants, osmotic protectant, *etc.*) that help to prevent injury and/or stress to the cells to be transplanted.

[0041] The composition of lipoic acid used in the present invention is typically pharmaceutical grade material for use in humans and/or other animals. In certain embodiments, the lipoic acid is approved for use in humans and for veterinary use. In some embodiments, the lipoic acid is approved by United States Food and Drug Administration. In some embodiments, the lipoic acid meets the standards of the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), the British Pharmacopoeia, and/or the International Pharmacopoeia. In certain embodiments, the lipoic acid is at least 90% pure. In certain embodiments, the lipoic acid is at least 95% pure. In certain embodiments, the lipoic acid is at least 98% pure. In certain embodiments, the lipoic acid is at least 99% pure. In certain embodiments, the lipoic acid is at least 99.5% pure. In certain embodiments, the lipoic acid is at least 99.9% pure. In certain embodiments, the lipoic acid is at least 99.99% pure. In certain embodiments, the lipoic acid is free of toxic or non-biocompatible materials.
Lipoic acids and derivatives thereof may be tested for use in fat transplantation by mixing a test lipoic acid with fat cells to be transplanted and transplanting the resulting composition in a mouse or other rodent to determine over time the success of the fat implant. Fat implants may be evaluated by various biochemical and pathological measurements, for example, weight of the implant, volume of the implant, DNA content, assessing markers of apoptosis and/or cell death, assessing mitochondrial ATP levels, or real-time PCR to determine levels of leptin, PPARγ, or other markers. In certain embodiments, the testing is performed in nude mice. Lipoic acids may also be screened in vitro by mixing cells with lipoic acid or a derivative thereof and assaying the cells for markers of apoptosis or cell death, assaying the cells for toxicity, etc. In certain embodiments, the results using a test lipoic acid are compared to the results from a control. In certain embodiments, the control fat transplant is treated with normal saline.

The lipoic acid may be combined with other biologically active agents and/or pharmaceutically acceptable excipients to form a composition useful for adding to cells to be transplanted. Such biologically active agents may also work to prevent cell death in a fat graft. Excipients may be used to aid in mixing the lipoic acid with the cells to be transplanted or handling and storage of the resulting lipoic acid/cell composition.

Biologically active agents that may be added along with a lipoic acid to the cells to be transplanted include, but are not limited to, antioxidants, vitamins, membrane stabilizers, minerals, osmotic protectants, coenzymes, viscosity enhancers, hormones, and growth factors. Numerous mechanisms have been implicated in the cause of cell death in transplanted cells, for example, membrane disruption and free radical formation. Antioxidants such as lipoic acid may be used in fat transplantation to reduce free radical formation. Antioxidants scavenge free radicals and prevent damage caused by reactive oxygen species. In certain embodiments, a lipoic acid/cell composition further comprises an additional antioxidant. In certain embodiments, the lipoic acid and/or additional antioxidant improve protection of the cells and thereby improve fat grafting results. The additional antioxidants may be enzymatic or nonenzymatic antioxidants. Enzymatic antioxidants include, for example, superoxide dismutase, glutathione peroxidase, and catalase. Exemplary non-enzymatic antioxidants include ascorbic acid (vitamin C), alpha-tocopherol (vitamin E), vitamin A, glutathione, carotenoids (e.g., lycopene, lutein, polyphenols, β-carotene), flavonoids, flavones, flavonols, glutathione, N-acetyl cysteine, cysteine, ubiquinonal (coenzyme Q), ubiquinone (coenzyme Q10), melatonin, lycopene, butylated hydroxyanisole, butylated hydroxytoluene (BHT), benzoates, methyl paraben, propyl paraben, proanthocyanidins, mannitol, and ethylenediamine tetraacetic.
acid (EDTA). In certain embodiments, the antioxidant is a metallic antioxidant. In certain embodiments, the antioxidant is selenium. In certain embodiments, the antioxidant is zinc. In certain embodiments, the antioxidant is copper. In certain embodiments, the antioxidant is pyrithione.

In certain embodiments, the antioxidant is a metallic antioxidant. In certain embodiments, the antioxidant is selenium. In certain embodiments, the antioxidant is zinc. In certain embodiments, the antioxidant is copper. In certain embodiments, the antioxidant is pyrithione.

[0045] In certain embodiments, a lipoic acid/cell composition further comprises a vitamin. The vitamin may be an antioxidant. In certain embodiments, the vitamin is alpha-tocopherol (vitamin E). The term "tocotrienol" encompasses natural and/or synthetic counterparts of tocopherol (vitamin E) that bear unsaturated tails, and include, but not limited to, α-, β-, γ-, and δ-tocotrienols, tocotrienol P25, desmethyl-tocotrienol, didesmethyl-tocotrienol, their synthetic counterparts, their counterparts having methylated or demethylated chroman rings, and mixtures thereof. In certain embodiments, fat cells are combined with lipoic acid and vitamin E for transplantation into a subject. In certain embodiments, the vitamin is coenzyme Q10. In certain embodiments, the vitamin is beta-carotene. Other vitamins that may be added to the inventive lipoic acid/cell composition include vitamin A, vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B4 (adenine), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxine), vitamin B7 (biotin), vitamin B9 (folic acid), vitamin B12 (cyanocobalamin), vitamin D (ergocalciferol), and vitamin K.

[0046] In certain embodiments, a lipoic acid/cell composition further comprises a membrane stabilizer. The membrane stabilizer is thought to facilitate the sealing of cell membranes to prevent cellular injury. In certain embodiments, the membrane stabilizer is not a polymer.

[0047] In certain embodiments, a lipoic acid/cell composition further comprises an osmotic protectant. Such an osmotic protectant may aid in protecting the cells in the cell/lipoic acid composition from osmotic damage or osmotic stress. In certain embodiments, the osmotic protectant is a polysaccharide. In certain embodiments, the osmotic protectant is maltose. In certain embodiments, the osmotic protectant is raffinose. In certain embodiments, the osmotic protectant is sucrose. In certain embodiments, the osmotic protectant is mannitol. In certain embodiments, the osmotic protectant is PEG. In certain embodiments, the osmotic protectant is not a polymer.

[0048] In certain embodiments, a lipoic acid/cell composition further comprises a viscosity enhancer. In certain embodiments, the viscosity enhancer is a polymer. In certain embodiments, the viscosity enhancer is a polysaccharide. In certain embodiments, the viscosity enhancer is cellulose or a cellulose derivative. In certain embodiments, the viscosity enhancer is carboxymethylcellulose. In certain embodiments, the viscosity enhancer is methyl cellulose.
In certain embodiments, the viscosity enhancer is ethyl cellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxyethyl cellulose, hydroxyethylcellulose, hydroxypropylcellulose, or hydroxybutyl cellulose. In certain embodiments, the viscosity enhancer is a wax or fatty alcohol (e.g., cetyl alcohol). In certain embodiments, the viscosity enhancer is not a polymer.

In certain embodiments, a lipoic acid/cell composition further comprises an alcohol (e.g., polyphenols, fatty alcohol). In certain embodiments, a lipoic acid/cell composition further comprises a hormone or growth factor. In certain embodiments, the hormone or growth factor is insulin, glitazones, cholesterol, VEGF, FGF, EGF, PDGF, etc. In certain embodiments, the lipoic acid/cell composition further comprises a small organic molecule (e.g., anthocyanins, capsaicins). In certain embodiments, the lipoic acid/cell composition further comprises a steroidal compound (e.g., cholesterol). In certain embodiments, the lipoic acid/cell composition further comprises a lipid.

The formulations of the lipoic acids described herein may be prepared by any method known or hereafter developed in the art of pharmaceuticals. In general, such preparatory methods include the step of bringing the lipoic acid into association with one or more excipients and/or one or more other biologically active agents. The relative amounts of the lipoic acid, the pharmaceutically acceptable excipient(s), and/or any additional agents in a composition of the invention will vary, depending upon the identity of the lipoic acid (e.g., lipoic acid derivative), implantation site, and/or subject. By way of example, the composition to be mixed with cells to be transplanted may comprise between 0.0001% and 99% (w/w) of lipoic acid. In a specific example, lipoic acid may be mixed with the cells at a concentration of (w/lipoic acid/Wfat cells) 100 mg/kg or 0.01% of lipoic acid. In certain embodiments, the concentration of lipoic acid ranges from 0.0001% to 1.0%. In other embodiments, the concentration of lipoic acid ranges from 0.001% to 0.1%.

Formulations of lipoic acid may comprise a pharmaceutically acceptable excipient, which, as used herein, includes any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular formulation desired. Remington's The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro, (Lippincott, Williams & Wilkins, Baltimore, MD, 2006; incorporated herein by reference) discloses various excipients used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional excipient is incompatible with a substance or its derivatives, such as by producing
any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention.

[0052] In some embodiments, the pharmaceutically acceptable excipient is at least 95%, 96%, 97%, 98%, 99%, or 100% pure. In some embodiments, the excipient is approved for use in humans and for veterinary use. In some embodiments, the excipient is approved by United States Food and Drug Administration. In some embodiments, the excipient is pharmaceutical grade. In some embodiments, the excipient meets the standards of the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), the British Pharmacopoeia, and/or the International Pharmacopoeia.

[0053] Pharmaceutically acceptable excipients used in the manufacture of lipoic acid compositions include, but are not limited to, inert diluents, dispersing agents, surface active agents and/or emulsifiers, disintegrating agents, preservatives, buffering agents, lubricating agents, and/or oils. Such excipients may optionally be included in the inventive formulations. Excipients such as coloring agents can be present in the composition, according to the judgment of the formulator.

[0054] Exemplary diluents include, but are not limited to, calcium carbonate, sodium carbonate, calcium phosphate, dicalcium phosphate, calcium sulfate, calcium hydrogen phosphate, sodium phosphate lactose, sucrose, cellulose, microcrystalline cellulose, kaolin, mannitol, sorbitol, inositol, sodium chloride, dry starch, cornstarch, powdered sugar, etc., and combinations thereof.

[0055] Exemplary dispersing agents include, but are not limited to, potato starch, corn starch, tapioca starch, sodium starch glycolate, clays, alginic acid, guar gum, citrus pulp, agar, bentonite, cellulose and wood products, natural sponge, cation-exchange resins, calcium carbonate, silicates, sodium carbonate, cross-linked poly(vinyl-pyrrolidone) (crospovidone), sodium carboxymethyl starch (sodium starch glycolate), carboxymethyl cellulose, cross-linked sodium carboxymethyl cellulose (croskarmellose), methylcellulose, pregelatinized starch (starch 1500), microcrystalline starch, water insoluble starch, calcium carboxymethyl cellulose, magnesium aluminum silicate (Veegum), sodium lauryl sulfate, quaternary ammonium compounds, etc., and combinations thereof.

[0056] Exemplary surface active agents and/or emulsifiers include, but are not limited to, natural emulsifiers (e.g. acacia, agar, alginic acid, sodium alginate, tragacanth, chondrux, cholesterol, xanthan, pectin, gelatin, egg yolk, casein, wool fat, cholesterol, wax, and lecithin), colloidal clays (e.g. bentonite [aluminum silicate] and Veegum [magnesium aluminum
silicate]), long chain amino acid derivatives, high molecular weight alcohols (e.g. stearyl alcohol, cetyl alcohol, oleyl alcohol, triacetin monostearate, ethylene glycol distearate, glyceryl monostearate, and propylene glycol monostearate, polyvinyl alcohol), caromers (e.g. carboxy polymethylene, polyacrylic acid, acrylic acid lipoic acid, and carboxyvinyl lipoic acid), carrageenan, cellulosic derivatives (e.g. carboxymethylcellulose sodium, powdered cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose), sorbitan fatty acid esters (e.g. polyoxyethylene sorbitan monolaurate [Tween®20], polyoxyethylene sorbitan [Tween®80], polyoxyethylene sorbitan monoooleate [Tween®80], sorbitan monopalmitate [Span®40], sorbitan monostearate [Span®60], sorbitan tristearate [Span®65], glyceryl monooleate, sorbitan monooleate [Span®80]), polyoxyethylene esters (e.g. polyoxyethylene monostearate [Myrj®45], polyoxyethylene hydrogenated castor oil, polyethoxylated castor oil, polyoxyethylene stearate, and Solutol), sucrose fatty acid esters, polyethylene glycol fatty acid esters (e.g. Cremophor®), polyoxyethylene ethers, (e.g. polyoxyethylene laurel ether [Brij®30]), poly(vinyl-pyrrolidone), diethylene glycol monolaurate, triethanolamine oleate, sodium oleate, potassium oleate, ethyl oleate, oleic acid, ethyl laurate, sodium lauryl sulfate, cetrimonium bromide, cetylpyridinium chloride, benzalkonium chloride, docusate sodium, etc. and/or combinations thereof.

Exemplary preservatives may include antioxidants, chelating agents, antimicrobial preservatives, antifungal preservatives, alcohol preservatives, acidic preservatives, and other preservatives. Exemplary antioxidants include, but are not limited to, alpha tocopherol, ascorbic acid, acorbil palmitate, butylated hydroxyanisole, butylated hydroxytoluene, monothioglycerol, potassium metabisulfite, propionic acid, propyl gallate, sodium ascorbate, sodium bisulfite, sodium metabisulfite, and sodium sulfate. Exemplary chelating agents include ethylenediaminetetraacetic acid (EDTA), citric acid monohydrate, disodium edetate, dipotassium edetate, edetic acid, fumaric acid, malic acid, phosphoric acid, sodium edetate, tartaric acid, and trisodium edetate. Exemplary antimicrobial preservatives include, but are not limited to, benzalkonium chloride, benzethonium chloride, benzyl alcohol, bronopol, cetrimide, cetylpyridinium chloride, chlorhexidine, chlorobutanol, chlorocresol, chloroxylenol, cresol, ethyl alcohol, glycerin, hexetidine, imidurea, phenol, phenoxyethanol, phenylethyl alcohol, phenylmercuric nitrate, propylene glycol, and thimerosal. Exemplary antifungal preservatives include, but are not limited to, butyl paraben, methyl paraben, ethyl paraben, propyl paraben, benzoic acid, hydroxybenzoic acid, potassium benzoate, potassium sorbate, sodium benzoate, sodium propionate, and sorbic acid. Exemplary alcohol preservatives include, but are not limited to, ethanol, polyethylene glycol, phenol, phenolic compounds, bisphenol,
chlorobutanol, hydroxybenzoate, and phenylethyl alcohol. Exemplary acidic preservatives include, but are not limited to, vitamin A, vitamin C, vitamin E, beta-carotene, citric acid, acetic acid, dehydroacetic acid, ascorbic acid, sorbic acid, and phytic acid. Other preservatives include, but are not limited to, tocopherol, tocopherol acetate, dextrose mesylate, cetrimide, butylated hydroxyanisol (BHA), butylated hydroxytoluened (BHT), ethylenediamine, sodium lauryl sulfate (SLS), sodium lauryl ether sulfate (SLES), sodium bisulfite, sodium metabisulfite, potassium sulfite, potassium metabisulfite, Glydant Plus®, Phenonip®, methylparaben, Germall 115, Germaben II, Neolone™, Kathon™, and Euxyl®. In certain embodiments, the preservative is an antioxidant. In other embodiments, the preservative is a chelating agent.

[0058] Exemplary buffering agents include, but are not limited to, citrate buffer solutions, acetate buffer solutions, phosphate buffer solutions, ammonium chloride, calcium carbonate, calcium chloride, calcium citrate, calcium glubionate, calcium gluceptate, calcium gluconate, D-gluconic acid, calcium glycerophosphate, calcium lactate, propanoic acid, calcium levulinate, pentanoic acid, dibasic calcium phosphate, phosphoric acid, tribasic calcium phosphate, calcium hydroxide phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dibasic potassium phosphate, monobasic potassium phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, dibasic sodium phosphate, monobasic sodium phosphate, sodium phosphate mixtures, tromethamine, magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, etc., and combinations thereof.

[0059] Exemplary lubricating agents include, but are not limited to, magnesium stearate, calcium stearate, stearic acid, silica, talc, malt, glyceryl behenate, hydrogenated vegetable oils, polyethylene glycol, sodium benzoate, sodium acetate, sodium chloride, leucine, magnesium lauryl sulfate, sodium lauryl sulfate, etc., and combinations thereof.

[0060] Exemplary oils include, but are not limited to, almond, apricot kernel, avocado, babassu, bergamot, black current seed, borage, cade, camomile, canola, caraway, carnauba, castor, cinnamon, cocoa butter, coconut, cod liver, coffee, corn, cotton seed, emu, eucalyptus, evening primrose, fish, flaxseed, geraniol, gourd, grape seed, hazel nut, hyssop, isopropyl myristate, jojoba, kukui nut, lavandin, lavender, lemon, litsea cubeba, macadamia nut, mallow, mango seed, meadowfoam seed, mink, nutmeg, olive, orange, orange roughy, palm, palm kernel, peach kernel, peanut, poppy seed, pumpkin seed, rapeseed, rice bran, rosemary, safflower, sandalwood, sasquana, savoury, sea buckthorn, sesame, shea butter, silicone,
soybean, sunflower, tea tree, thistle, tsubaki, vetiver, walnut, and wheat germ oils. Exemplary oils include, but are not limited to, butyl stearate, caprylic triglyceride, capric triglyceride, cyclomethicone, diethyl sebacate, dimethicone 360, isopropyl myristate, mineral oil, octyldecaneol, oleyl alcohol, silicone oil, and combinations thereof.

[0061] The inventive lipoic acid besides being combined with the cells to be transplanted may be combined with one or more biologically active agents. In certain embodiments, the lipoic acid is combined with an additional antioxidant. In certain embodiments, the lipoic acid is combined with a vitamin. In certain embodiments, the lipoic acid is combined with a lipid. In certain embodiments, the lipoic acid is combined with a membrane stabilizer. In certain embodiments, the lipoic acid is combined with a pharmaceutical agent. In certain embodiments, the lipoic acid is combined with an anti-inflammatory agent. In certain embodiments, the lipoic acid is combined with an antibiotic. In certain embodiments, the lipoic acid is combined with a protein or peptide. In certain embodiments, the lipoic acid is combined with a hormone. In certain embodiments, the lipoic acid is combined with a growth factor. In certain embodiments, the lipoic acid is combined with a carbohydrate. In certain embodiments, the lipoic acid is combined with a liquid excipient for administering the lipoic acid/cell composition. In certain embodiments, the excipient is an aqueous solution. In certain embodiments, the excipient is a buffered aqueous solution. In certain embodiments, the excipient is phosphate-buffered saline solution. In certain embodiments, the excipient is isotonic with extracellular fluid.

Uses

[0062] The invention provides methods of associating lipoic acid or lipoic acid derivatives with cells or tissue to be transplanted before transplantation. The inventive system is particularly useful in improving the success of fat transplantation or improving the success of the transplantation of cells derived from adipose tissue. Cells or tissue to be transplanted are mixed with lipoic acid or a derivative thereof at a sufficient concentration to provide beneficial effects that support cell survival (e.g., by reducing cellular injury and/or cell stress) during cell harvesting, cell storage, cell handling, and/or cell transplantation. In certain embodiments, lipoic acid is mixed to the cells or tissue at a sufficient concentration to provide antioxidant effects thereby reducing inflammation and/or oxidative stress. In certain embodiments, lipoic acid is mixed to the cells at a sufficient concentration to provide stabilizing effects to mitochondrial function, glucose transport, and/or insulin sensitivity to allow for graft
stabilization while a neovascular network is established. In certain embodiments, lipoic acid treatment significantly reduces long-term reabsorption in free fat grafting.

[0063] The cells or a composition of cells are mixed with lipoic acid or a composition comprising lipoic acid or a derivative thereof before transplantation into a subject. Lipoic acid or a derivative thereof may be mixed with the cells at the time of procurement of the cells, during the storage or handling of the cells, or just prior to implantation of the cells into a subject.

[0064] In certain embodiments, the cells to be transplanted are contacted with lipoic acid or a derivative thereof as soon as they are harvested to protect them from damage. For example, after fat tissue is harvested from a donor (e.g., by liposuction or by a needle aspirate), it may be immediately contacted with lipoic acid or a derivative thereof. In certain embodiments, the cells to be transplanted are harvested from the same person receiving them (i.e., an autologous donation). In certain embodiments, the cells are harvested from the stomach, thigh, or buttocks of the donor. In certain embodiments, the fat tissue is harvested into a syringe or other container that already includes lipoic acid or a composition of lipoic acid or a derivative thereof. In other embodiments, the fat tissue is harvested and immediately added to a composition of the lipoic acid, or a composition of lipoic acid is added to the fat tissue. The resulting lipoic acid/cell composition may be further processed before implantation into a subject. For example, the cells may be washed, purified, extracted, or otherwise treated before implantation into a subject.

[0065] In certain embodiments, the cells to be transplanted are contacted with lipoic acid or a derivative thereof immediately before transplantation. For example, the cells may be mixed with lipoic acid or a derivative thereof in the operating room or clinic just prior to implantation into a subject. The sterile lipoic acid or a derivative thereof or composition thereof is mixed with the cells to be transplanted.

[0066] The cells or tissue is typically mixed with lipoic acid at a concentration (w/w per fat cells) ranging from approximately 1 mg/kg to about 10 g/kg. In a specific example, lipoic acid may be mixed with the cells or tissue at a concentration of 100 mg/kg of lipoic acid. As would be appreciated by one of skill in the art, the concentration of lipoic acid needed to provide the beneficial effects described herein (e.g., anti-oxidant, anti-inflammatory effects, and/or stabilizing effects to mitochondrial function, glucose transport, and/or insulin sensitivity) to the cells or tissue to be transplanted may vary depending on the lipoic acid used (e.g., lipoic acid derivative), the subject, the site of implantation, etc. In certain embodiments, the concentration ranges from approximately 10 mg to about 1 g of lipoic acid per kg of cells or
tissue or the concentration ranges from approximately 50 mg to about 200 mg of lipoic acid per kg of cells or tissue.

[0067] After the cells or tissue are contacted with lipoic acid, the cell/lipoic acid composition is administered to a subject. In certain embodiments, the subject is a human. In certain embodiments, the subject is a mammal. In certain embodiments, the subject is a test animal such as a mouse, rat, rabbit, or dog. The cell/lipoic acid composition is typically administered to a patient in need of a fat transplant. The subject may be undergoing reconstructive or cosmetic surgery. In certain embodiments, the fat transplantation is used in removing wrinkles. In certain embodiments, fat transplantation is used in soft tissue replacement or augmentation. In certain embodiments, fat transplantation is used in augmentation of the lips, cheeks, breasts, face, buttocks, calves, pectorals, and penis. Typically, autologous fat cells are transplanted back into the donor at a different site from which the cells were taken.

[0068] Besides adipocytes, fat tissue has been found to be a source of stem cells (Gimble et al., "Adipose-Derived Stem Cells for Regenerative Medicine" Circulation Research 100:1249-1260, 2007; incorporated herein by reference). Therefore, the inventive system may be useful in stabilizing and/or preventing damage to stem cells or other cells derived from fat tissue. In certain embodiments, the inventive system is useful in the transplantation of adult stem cells. In certain embodiments, the inventive system is useful in the transplantation of fibroblasts. In certain embodiments, the inventive system is useful in the transplantation of other mammalian cell types, for example, keratinizing epithelial cells (e.g., epidermal keratinocyte; epidermal basal cell; medullary, cortical, cuticular hair shaft cell; hair root sheath cell, hair matrix cell); exocrine secretory epithelial cells (e.g., salivary gland mucous cell; salivary gland serous cell; Von Ebner's gland cell; mammary gland cell; lacrimal gland cell; ceruminous gland cell; eccrine sweat gland dark or clear cell; apocrine sweat gland cell; gland of Moll cell; sebaceous gland cell; Bowman's gland cell; Brunner's gland cell; seminal vesicle cell; prostate gland cell; Bulbourthral gland cell; Bartholin's gland cell; Gland of Littre cell; uterine endometrium cell; isolated goblet cell of respiratory and digestive tracts; stomach lining mucous cell, gastric gland zymogenic cell; gastric gland oxyntic cell; pancreatic acinar cell; Paneth cell of small intestine; Type II pneumocyte; Clara cell); hormone secreting cells (e.g. anterior pituitary cells: somatotropes, lactotropes, thyrotropes, gonadotropes; corticotropes; intermediate pituitary cell; magnocellular neurosecretory cells; gut and respiratory tract cells; thyroid gland cells; thyroid epithelial cell; parafollicular cell; parathyroid gland cells; parathyroid chief cell; oxyphil cell, adrenal gland cells; chromaffin cells; Leydig cell; theca interna cell; juxtaglomerular cell;
macula densa cell; peripolar cell; mesangial cell); kidney cells (e.g., glomerulus parietal cell, glomerulus podocyte, proximal tubule brush border cell, loop of Henle thin segment cell, distal tubule cell, collecting duct cell); barrier function cells (e.g., type I pneumocyte; pancreatic duct cell; nonstriated duct cell; principal cell; intercalated cell; duct cell; intestinal brush border cell; exocrine gland striated duct cell; gall bladder epithelial cell; ductulus efferens nonciliated cell; epididymal principal cell; epididymal basal cell); lining epithelial cells (e.g., blood vessel and lymphatic vascular endothelial fenestrated cell; blood vessel and lymphatic vascular endothelial continuous cell; blood vessel and lymphatic vascular endothelial splenic cell; synovial cell; serosal cell; squamous cell; columnar cell; dark cell; vestibular membrane cell; stria vascularis basal cell; stria vascularis marginal cell; cell of Claudius; cell of Boettcher; choroid plexus cell; pia-arachnoid squamous cell; pigmented ciliary epithelium; nonpigmented ciliary epithelium cell; corneal endothelial cell); extracellular matrix secretion cells (e.g., ameloblast epithelial cell; planum semilunatum epithelial cell; organ of Corti interdental epithelial cell; loose connective tissue fibroblasts; corneal fibroblasts; tendon fibroblasts; bone marrow reticular tissue fibroblastst; pericyte; nucleus pulposus cell of intervertebral disc; cementoblast/cementocyte; odontoblast/odontocyte; hyaline cartilage chondrocyte; fibrocartilage chondrocyte; elastic cartilage chondrocyte; osteoblast/osteocyte; osteoprogenitor cell; hylarocyte; stellate cell; hepatic stellate cell (Ito cell); pancreatic stellate cell); contractile cells (e.g., skeletal muscle cells; red skeletal muscle cell; white skeletal muscle cell; intermediate skeletal muscle cell; nuclear bag cell; nuclear chain cell; satellite cell; heart muscle cells: nodal heart muscle cell, purkinje fiber cell; smooth muscle cell; myoepithelial cell); blood and immune system cells (e.g., erythrocyte; megakaryocyte; monocyte; macrophage; epidermal Langerhans cell osteoclast; dendritic cell; microglial cell; neutrophil granulocyte; eosinophil granulocyte; basophil granulocyte; mast cell; helper T cell; suppressor T cell; cytotoxic T cell; natural Killer T cell; B cell; reticulocyte); sensory transducer cells (e.g., auditory inner and outer hair cell of organ of Corti; basal cell of olfactory epithelium; cold- or heat-sensitive primary sensory neurons; Merkel cell; olfactory receptor neuron; pain-sensitive primary sensory neurons; photoreceptor cells of retina in eye: rod cells, blue-, green-, red-sensitive cone cell; proprioceptive primary sensory neurons; touch-sensitive primary sensory neurons; type I and II carotid body cell; type I and II hair cell; type I taste bud cell; cholinergic neural cell; adrenergic neural cell; peptidergic neural cell; inner and outer pillar cell of organ of Corti; inner and outer phalangeal cell of organ of Corti; border cell of organ of Corti; Hensen cell of organ of Corti; vestibular apparatus supporting cell; type I taste bud supporting cell; olfactory epithelium supporting cell; Schwann cell; satellite cell; enteric glial cell;
astrocyte; oligodendrocyte; neurons; spindle neuron; lens cells: anterior lens epithelial cell, crystallin-containing lens fiber cell); pigment cells (e.g., melanocyte; retinal pigmented epithelial cell); germ cells (e.g., oogonium/Oocyte; spermatid; spermatocyte; spermatogonium cell; spermatozoon); nurse cells (e.g., ovarian follicle cell, Sertoli cell).

**Kits**

[0069] The invention, in certain embodiments, also provides packages or kits, comprising one or more lipoic acids (and derivatives thereof) as described herein in a container. For example, the container may include lipoic acid or composition of lipoic acid ready for use in fat transplantation. The package can also include a notice associated with the container, typically in a form prescribed by a government agency regulating the manufacture, use, or sale of medical devices and/or pharmaceuticals, whereby the notice is reflective of approval by the agency of the compositions, for human or veterinary administration in tissue transplantation. Instructions for the use of lipoic acid may also be included. Such instructions may include information relating to administration of lipoic acid or a lipoic acid/cell composition to a patient. In particular, the instructions may include information regarding the contacting of lipoic acid with cells and administration of the cell/lipoic acid composition to a patient. The package may also include one or more containers containing biologically active agent(s) to be included in the lipoic acid/cell composition prior to administration.

[0070] The package may include a device or receptacle for preparation of the lipoic acid/cell composition. The device may be, e.g., a measuring or mixing device.

[0071] The package may also optionally include a device for administering a lipoic acid/cell composition of the invention. Exemplary devices include specialized syringes, needles, and catheters that are compatible with a variety of laryngoscope designs.

[0072] The components of the kit may be provided in a single larger container, e.g., a plastic or styrofoam box, in relatively close confinement. Typically, the kit is conveniently packaged for use by a health care professional. In certain embodiments, the components of the kit are steriley packaged for use in a sterile environment such as an operating room or physician's office.

**Examples**

**Methods**

[0073] Apoptosis: Immunochemistry apoptosis specific fluorescent labels FLIVO™ and MT Mito™ were used to identify apoptosis in adipocytes. Fat grafts were explanted, treated...
with Blendzyme (Roche Applied Science Liberase Blendzyme 3) at 38° C for 20 minutes, and passed through a 100 micron cell strainer (to remove extra-cellular stromal elements left behind after digestion). These cells were then incubated for one hour at 38° C, and washed once following labeling. Afterwards these cells were placed into 96 well black plates, and read on a plate reader (Molecular Devices, SpectraMax M2) at their dye-specific emission and excitation spectra (FLIVO-red ex 565 / em >590, MITO-PT ex 488 / em green 530, red 590).

[0074] PICO green: Quant-iT PicoGreen® dsDNA Assay Kit from Invitrogen was used for quantification of DNA as a surrogate for cell counts. Each explanted lobule was digested in 1.Occ of Blendzyme at 38° C for 30 minutes. Next, the Blendzyme reaction was stopped with 1.Occ of FBS containing cell-culture media. For quantification 75 micro-liters of digested fat was removed and processed using a Qiagen Mini-prep spin column isolation kit. Extracted DNA samples were then incubated with reagents A (PicoGreen® fluorophore), B (Tris buffer), and for standards with component C (Lambda dsDNA standards, 100 mcg/ml concentration), according to kit protocol. These samples were then read on a plate reader, using a black 96 well plate.

[0075] ATP Activity: ATP levels were assayed using the Perkin Elmer DNA-Lite kit. Samples were digested as above and 100 μl of digested fat was assayed in triplicate for each sample on a white clear bottom Costar 96-well plate. The fat was assayed according to the kit protocol and read on a Molecular Devices, SpectraMax M2, luminescent plate reader.

[0076] Fat Processing: Liposuction was taken directly from the operating room. It was separated into 30cc aliquots, and washed once with an equal volume of normal saline to remove blood and cellular debris. Afterwards, the tubes were centrifuged at 200G and the middle layer was separated and treated with various agents. These agents were treated in 30 cc washes, with 30 cc of fat for 30 minutes at 37° C. Following incubation and washing, the fat was again centrifuged at 200G. The middle fat layer was again separated and placed in 1.0 cc, 1.0 g aliquots for injection into the flanks of nude mice.

[0077] Mouse Experimental Model : Nude mice were implanted with 1.0 cc fat grafts in a single lobule (see Figure 1). These were injected with a 14ga angio-catheter to simulate a large-volume injection. Initially, the lobules were explanted daily and measured for weight and apoptotic activity. After early curves were generated, lobules were explanted on days 3, 6, and 9, for the primary measurement of apoptosis levels. Additionally these samples were weighed, sent for histology, and measured for DNA content. Endpoints in the first ten days were compared to endpoints at six weeks to determine whether graft performance can be accurately predicted by early apoptosis and cell-death.
Lipoic acid: Lipoic acid was tested at 100 mg/kg dose for 30g of treated fat, in a 30cc normal saline wash for 20 minutes. Normal saline was used as a control wash.

Statistics: Samples were analyzed using ANOVA for statistical significance between groups. Significance was set at a 95% confidence interval.

Results

Weights: The average ten day weights in this study were: saline 0.76 g +/- 0.09 g; and lipoic acid 0.72g +/- 0.09g (1.0g +/- 0.10g initial implant). Treatment groups were then reexamined at 6 weeks; here significant differences were noted in the weights. The average weights per group at six weeks were: saline 0.58g +/- 0.07g; lipoic acid 0.65 g +/- 0.09g. In comparing early to late changes, re-absorption percentages were calculated using the dehydrated weight of the samples over the first ten days, to six week final weight. Lipoic acid demonstrated statistically significant differences in reabsorption (p-value <0.05), as compared to saline (see Figure 1).

Apoptosis: Saline treated fat demonstrated the highest level of apoptosis (863 RFU) on day 6. Lipoic acid treated samples demonstrated the lowest level on day 6 (772 RFU and 625 RFU) with p-value <0.05 (see Figure 2). Additionally, lipoic acid demonstrated, on average, the lowest level of apoptosis over the first ten days when compared to saline (462 vs. 601 respectively).

DNA Content: Explanted samples were assayed for DNA content, as an indirect measure of cell count. Digested samples were studied in both the early and late time-points. Similar to the weight measurements, there were no significant differences in DNA content between samples in the first 10 days. At six weeks DNA content was the following: saline 0.00138#mcg +/- 0.00021; lipoic acid 0.0014mcg +/- 0.00023 (see Figure 3, p< 0.05).

ATP Activity: Raw ATP luminescence levels were as follows: saline 18.6 RLU; LA 50.9 RLU, (see Figure 4). These differences represent a significant difference in cellular ATP activity between samples at six weeks.

Histology: Samples were sent for histology and H&E staining at 6 weeks. H&E staining demonstrated dramatic qualitative differences between lipoic acid, and saline treated samples (see Figure 5). In saline-treated controls, large amounts of fibrosis were noted throughout explanted lobules. The normal adipose architecture was disorganized with large vacuoles present. Conversely, lipoic acid-treated fat appeared architecturally normal.

References


**Equivalents and Scope**

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the above description, but rather is as set forth in the appended claims.

In the claims articles such as "a," "an," and "the" may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention also includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process. Furthermore, it is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the claims or from relevant portions of the description is introduced into another claim. For example, any claim that is dependent on another claim can be modified to include one or more limitations found in any other claim that is dependent on the same base claim. Furthermore, where the claims recite a composition, it is to be understood that methods of using the composition for any of the purposes disclosed herein are included, and methods of making the composition according to any of the methods of making disclosed herein or other methods known in the art are included, unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. For example, it is to be understood that any of the compositions of the invention can be used for soft tissue repair or augmentation. It is also to be
understood that any of the compositions made according to the methods for preparing compositions disclosed herein can be used for soft tissue repair or augmentation. In addition, the invention encompasses compositions made according to any of the methods for preparing compositions disclosed herein.

Where elements are presented as lists, e.g., in Markush group format, it is to be understood that each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It is also noted that the term "comprising" is intended to be open and permits the inclusion of additional elements or steps. It should be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements, features, steps, etc., certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements, features, steps, etc. For purposes of simplicity those embodiments have not been specifically set forth in haec verba herein. Thus for each embodiment of the invention that comprises one or more elements, features, steps, etc., the invention also provides embodiments that consist or consist essentially of those elements, features, steps, etc.

Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and/or the understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise. It is also to be understood that unless otherwise indicated or otherwise evident from the context and/or the understanding of one of ordinary skill in the art, values expressed as ranges can assume any subrange within the given range, wherein the endpoints of the subrange are expressed to the same degree of accuracy as the tenth of the unit of the lower limit of the range.

In addition, it is to be understood that any particular embodiment of the present invention may be explicitly excluded from any one or more of the claims. Any embodiment, element, feature, application, or aspect of the compositions and/or methods of the invention, can be excluded from any one or more claims. For purposes of brevity, all of the embodiments in which one or more elements, features, purposes, or aspects is excluded are not set forth explicitly herein.
Claims

What is claimed is:

1. A method for transplanting fat-derived cells or adipose tissue, the method comprising:
   administering a composition of fat-derived cells or adipose tissue, and a lipoic acid or a
   derivative thereof to a subject, wherein the lipoic acid protects the cells from injury and
   prevents cell death.

2. The method of claim 1, wherein the lipoic acid provides an anti-oxidant effect to the
   cells.

3. The method of claim 1, wherein the lipoic acid reduces an inflammatory response.

4. The method of claim 1, wherein the lipoic acid stabilizes mitochondrial function of the
   cells.

5. The method of claim 1, wherein the lipoic acid stabilizes glucose transport of the cells.

6. The method of claim 1, wherein the lipoic acid maintains insulin sensitivity of the cells.

7. The method of claim 1, wherein the fat-derived cells are adipocytes.

8. The method of claim 1, wherein the fat-derived cells are stem cells.

9. The method of claim 1, wherein the fat-derived cells are autologous cells.

10. The method of claim 1, wherein the lipoic acid derivative is selected from the group
    consisting of thioctic acid ester, thioctic acid amide, thioctic acid lipoamid, thioctic acid salt,
    thioctic acid anhydride, dihydrolipoic acid, dihydrolipoic acid ester, dihydrolipoic acid amide,
    dihydrolipoic acid salt, and dihydrolipoamide.

11. The method of claim 10, wherein the thioctic acid ester is a C₂ to C₂₄ alkyl esters.

12. The method of claim 10, wherein the thioctic acid ester is a C₄ to C₁₈ fatty acid esters.
13. The method of claim 10, wherein the thioctic acid lipoamid is DL-6,8-thioctic acid amide.

14. The method of claim 10, wherein the dihydrolipoic acid ester is a C_4 to C_{18} fatty acid esters.

15. The method of claim 10, wherein the dihydrolipoamide is a DL-lipoamide.

16. The method of claim 10, wherein the dihydrolipoic acid is dihydrolipoic acid N,N-dimethyl,N-2-amidoethyl lipoate.

17. The method of claim 1, wherein the composition of cells and lipoic acid further comprises an additional antioxidant.

18. The method of claim 17, wherein the antioxidant is selected from the group consisting of superoxide dismutase, glutathione peroxidase, and catalase.

19. The method of claim 17, wherein the antioxidant is selected from the group consisting of ascorbic acid, alpha-tocopherol, glutathione, carotenoids, N-acetyl cysteine, and flavonoids.

20. The method of claim 17, wherein the antioxidant is selected from the group consisting of selenium and zinc.

21. The method of claim 1, wherein the composition of cells and lipoic acid further comprises a vitamin.

22. The method of claim 21, wherein the vitamin is selected from the group consisting of vitamin E, vitamin C, vitamin A, beta-carotene, and coenzyme Q10.

23. The method of claim 1, wherein the composition of cells and lipoic acid further comprises an osmotic protectant.
24. The method of claim 1, wherein the composition of cells and lipoic acid further comprises a carbohydrate.

25. The method of claim 24, wherein the carbohydrate is maltose.

26. The method of claim 24, wherein the carbohydrate is cellulose or a derivative of cellulose.

27. The method of claim 24, wherein the carbohydrate is carboxymethyl cellulose or methylcellulose.

28. The method of claim 1, wherein the composition of cells further comprises hormones and growth factors.

29. The method of claim 28, wherein the hormone or growth factor is selected from the group consisting of insulin, glitazones, and VEGF.

30. The method of claim 1, wherein the composition of cells and lipoic acid further comprises an alcohol.

30. The method of claim 1, wherein the composition of cells and lipoic acid further comprises a polyphenol.

31. The method of claim 1, wherein the composition of cells and lipoic acid further comprises an organic acid.

32. The method of claim 1, wherein the composition of cells and lipoic acid further comprises anthocyanins, and capscaisins.

33. The method of claim 1, wherein the lipoic acid is at least 95% pure.

34. The method of claim 1, wherein the lipoic acid is at least 98% pure.

35. The method of claim 1, wherein the lipoic acid is at least 99% pure.
36. The method of claim 1, wherein the subject is mammal.

38. The method of claim 1, wherein the subject is human.

39. A method of preventing the resorption of a fat graft comprising administering a composition of fat-derived cells and a lipoic acid or a derivative thereof to a subject.

40. The method of claim 39, wherein the cells are adipocytes.

41. The method of claim 39, wherein the cells are stem cells.

42. The method of claim 39, wherein the lipoic acid derivative is selected from the group consisting of thioctic acid ester, thioctic acid amide, thioctic acid lipoamid, thioctic acid salt, thioctic acid anhydride, dihydrolipoic acid, dihydrolipoic acid ester, dihydrolipoic acid amide, dihydrolipoic acid salt, and dihydrolipoamide.

43. A composition comprising fat-derived cells and a lipoic acid or derivative thereof.

44. The composition of claim 43, wherein the fat-derived cells are adipocytes.

45. The composition of claim 43, wherein the fat-derived cells are stem cells.

46. The composition of claim 43, wherein the fat-derived cells are a combination of adipocytes and stem cells.

47. The composition of claim 43, wherein the lipoic acid derivative is selected from the group consisting of thioctic acid ester, thioctic acid amide, thioctic acid lipoamid, thioctic acid salt, thioctic acid anhydride, dihydrolipoic acid, dihydrolipoic acid ester, dihydrolipoic acid amide, dihydrolipoic acid salt, and dihydrolipoamide.

48. The composition of claim 43, wherein adipocytes are at least 80% of the cells in the composition.
49. The composition of claim 43, wherein adipocytes are at least 90% of the cells in the composition.

50. The composition of claim 43, wherein adipocytes are at least 95% of the cells in the composition.

51. A method of identifying a lipoic acid derivative useful in fat transplantation, the method comprising steps of:
   contacting fat cells with a test lipoic acid at one or more different concentrations; and
   assaying for cell death or toxicity.

52. A method of identifying a derivative of lipoic acid useful in fat transplantation, the method comprising steps of:
   contacting fat cells with a test derivative of lipoic acid at one or more different concentrations;
   transplanting the cell/lipoic acid derivative composition into a test animal; and
   assessing the success of the transplantation.

53. The method of claim 52, wherein the test animal is a rodent.

54. The method of claim 52, wherein the test animal is a nude mouse.

55. The method of claim 52, wherein the step of assessing comprises assessing the transplanted cells by weight, volume, extent of cell death, mitochondrial levels, markers of apoptosis, DNA levels, ATP levels, or levels of leptin or PPARy2.
Percent Re-Absorption By Weight at 6 Weeks (With High/Low Removed)

Figure 1

Apoptosis as a Function of Time after Treatment and Implantation (1.0g/1.0cc)

Figure 2
ATP Activity 6 Weeks Post-Treatment

Figure 3

DNA Content in Micrograms at Six-weeks Post-Treatment

Figure 4
Saline 40x 6weeks

Lipoic Acid 40x 6weeks

Figure 5