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(54) Benævnelse: **Lipofyldning med ex-vivo-ekspanderede, fra fedtvæv stammende stamceller, til kosmetisk brystfyldning eller til ansigtsfyldning og/eller -foryngelse**

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DESCRIPTION

Field of the invention

[0001] The present invention relates to a composition for use in soft tissue filling, said composition comprising ex-vivo expanded adipose tissue-derived stem cell enriched fat grafts. These may be used for cosmetic breast filling/augmentation or for facial filling and/or rejuvenation.

Description of the invention

[0002] A frequent challenge in plastic surgery is the correction of volume defects and augmentation of existing volume. When correcting volume defects or when augmenting existing tissue, it is often necessary to use filling material, a so-called filler or an implant. Autologous fat grafting (lipofilling) enables repair and augmentation of soft tissues (e.g. cosmetic breast augmentation) and is increasingly used both in aesthetic and reconstructive surgery. Autologous fat tissue has been considered to be an ideal filler for augmentation of soft tissue because it is biocompatible, versatile, natural-appearing, non-immunogenic, inexpensive, and readily obtainable with low donor site morbidity [1,2]. The transplanted fat graft, however, has an unpredictable and often low survival, which is why investigators have sought new ways of increasing its viability. One animal study investigating xenogeneic fat grafting enriched with adipose tissue-derived mesenchymal stem cells (ASC) has indicated that the technique is valid and reproducible, and results in increased residual volume of the transplant compared to non-enriched [3]. A recent human study has demonstrated and confirmed the advantage and striking effect of adding ASCs to the fat graft for increased residual volume and better quality of the transplanted tissue [4].

[0003] In the cosmetic industry, the solutions available for cosmetic facial filling/rejuvenation are predominantly artificial (e.g. Botulinum Toxin Type A, Hyaluronic, Collagen, Calcium Hydroxylapatite, Polyactic acid, Polymethyl-methacrylate microspheres). Therefore, results often end up looking un-natural due to low versatility and bio-compatibility. The artificial fillers de-compose over time with possible adverse physical side-effects ranging from various sickness symptoms to beauty flaws. Autologous fat tissue is considered to be an ideal filler solution as described above. However, the outcomes for the patients treated with autologous fat for facial filling/rejuvenation (i.e. without stem cell enrichment) may end up with disproportionate results, due to the unpredictable survival of the graft.

[0004] Some surgeons propose a better and more predictable graft take can be accomplished by adding the so-called stromal vascular fraction (SVF) to the transplant [5,6]. The SVF is the cell pellet that forms when adipose tissue is harvested by liposuction and the fat cells are enzymatically digested using collagenase. The SVF is known to contain a small amount of adipose tissue-derived stem cells (ASCs).

[0005] DE 10 2011 121982 B3 describes the use of expanded adipose tissue derived stem cells to reconstruct skin defects.

[0006] It is important to distinguish between the following terms:

1. 1) Conventional lipofilling: Fat only
2. 2) Cell-assisted lipotransfer: Fat + SVF
3. 3) Stem cell enriched lipofilling: Fat + ex-vivo expanded ASCs
4. 4) Stem cell filling: ex-vivo expanded ASCs

[0007] In the current patent application, we only refer to terms 3) and 4 above.

[0008] Definition of the stem cells referred to in the current patent application:

Cells adherent to a culture surface when seeding and culturing the stromal vascular fraction.

[0009] For both breast and facial applications autologous or allogeneic cells may be used.

[0010] The present invention is especially suitable for cosmetic facial filling and rejuvenation and for cosmetic breast filling/augmentation.

Detailed description of the invention

[0011] The present invention relates to a composition comprising ex-vivo expanded adipose tissue-derived stem cells (ASC) mixed with harvested fat tissue at a ratio of 20 x 10^6 - 20 x 10^7 ASCs/mL fat and the use of ex-vivo expanded adipose tissue-derived stem cell enriched fat grafts as an agent for cosmetic breast filling/augmentation or for facial filling/rejuvenation.

Surgical procedure for harvesting ASCs

[0012] Patients receive an outpatient minor liposuction.

[0013] Lipoaspirates will be harvested by standard sterile liposuction techniques. Through incisions a wetting solution is infiltrated into the subcutaneous fat. The lipoaspirate is procured with a standard liposuction device (e.g. Vibrasat®) and sealed in a sterile container. The lipoaspirate is transported to the clinical stem cell laboratory.

Isolation and culture of ASCs

[0014] ASC isolation and ex vivo expansion will be performed in accordance with an approved protocol, in a laboratory approved for good manufacturing practice (GMP) and clinical stem cell expansion, at a Cell Therapy Facility.

[0015] The lipoaspirate is washed with phosphate-buffered saline (PBS) and centrifuged. To isolate the Stromal vascular fraction (SVF), the supernatant is incubated and enzymatically digested with collagenase (GMP grade). The enzymatic activity is neutralized using growth medium. The suspension is filtered using a 60-100 µm filter and centrifuged. The cell pellet is resuspended in culturing medium and the cells in the pellet that contain the SVF are counted. An alternative way of isolation the SVF is using a closed system e.g. the GID SVF-1™ system. The SVF is seeded in culture medium consisting of Dulbecco's modified Eagle's medium (DMEM) or Alpha minimal essential medium (α-MEM),1-5% penicillin-streptomycin, 1-5 IU/mL preservative-free heparin and 2-20% pooled Human Platelet Lysate pHPL or any other alternative growth medium e.g. Fetal Bovine Serum. The primary cultures (P0) are incubated. The non-adherent cells are discarded, the cell culture flasks are carefully rinsed with PBS, and the medium replaced. The medium is changed every 3-7 days. During culturing and on the day of ASC harvesting every culture flask/stack will be examined for pathogen contamination.

pHPL production

[0016] The pHPL may be manufactured as described by Schallmoser et al. [7], with minor modifications. Briefly, after informed consent, whole blood units are collected from healthy blood donors. All of the blood donations are tested for infectious disease markers, in adherence with existing law. The buffy coats are separated from the red blood cells and plasma. Four buffy-coat units are pooled with 1 unit of plasma into 1 unit of platelet-rich plasma (PRP) and are stored at -20°C to -80°C. Minimum ten units of PRP is thawed in a water bath and then pooled into a single PRP batch. The pooled PRP batch is aliquoted into smaller fractions and frozen at -20°C to -80°C. Next, all of the aliquoted bags from the single, pooled PRP batch are thawed in a water bath and centrifuged (e.g. at 4000 g for 15 min) to sediment the platelet fragments. Lastly, the pHPL-containing supernatant is transferred into new bags and stored at -40°C to -80°C for later use in the preparation of the cell culture medium.

[0017] ASCs are harvested in P0-P4. All cell culture flasks/stacks are washed with PBS and the cells are detached from the plastic surface using either chemical (e.g. Tryple Select) or physical processing. The suspension containing the ASCs is centrifuged (e.g. at 300 g for 5 min), the supernatant is removed and the cell pellet is collected after resuspension in PBS. The ASC cell pellet is washed with PBS, centrifuging the ASCs and discarding the supernatant after each washing procedure. Cells are counted three times and the average count will be calculated. ASCs will be carefully controlled before releasing them for clinical use, including 1) Absence of pathogen contamination 2) Viability of the ASCs greater than 90 % 3) Morphology, assessed to be characteristic for ASCs. The ASCs will be transported in approved sterile containers.

Liposuction, graft preparation and lipofilling procedure

[0018] The surgical procedure is conducted under either local or general anesthesia. Lipoaspirates are harvested by standard sterile liposuction techniques. Through incisions a wetting solution (e.g. Kleins solution) is infiltrated into the donor site using a blunt infiltrator. The harvesting cannula is 2-5 mm in diameter with a blunt tip, connected to a harvesting device (e.g. Vibrasat®). If needed the lipoaspirate is washed using saline. The harvested lipoaspirate is either left to sedimentate, spun or centrifuged (e.g. at 100 g for 5 min). After the separation procedure, the oil layer (upper level) is decanted and the aqueous layer (lower level) is also drained out of the syringes. The middle layer, composed of predominantly fat graft is used for transplantation.

[0019] The invented applications:

Cosmetic breast filling: The harvested fat tissue is mixed with the harvested ex-vivo expanded ASCs at a ratio of 20×10^6 - 20×10^7 ASCs/mL fat, and injected as aliquots into the breast for cosmetic augmentation.

[0020] Example of injection technique:

The enriched fat graft is injected to the breast using a long needle horizontally (parallel to the body) to avoid damaging structures outside the breast tissue. The needle is inserted from several points around the areola margin and at several points at the inframammary fold in variable directions and planes to achieve an even and natural appearing distribution of the graft.

[0021] For facial filling and wrinkle correction: The harvested fat tissue is mixed with the harvested ex-vivo expanded ASCs at a ratio of 20×10^6 - 20×10^7 ASCs/mL fat. The amount of stem cells will be increased depending on the amount of filling needed, the less filling is needed, the higher the concentration of ASCs. If the desired effect is purely a matter of tissue quality improvement, ASCs alone will be used, solved in PBS in order to evenly distribute the cells. Example of injection technique:

When used as a filler the fat grafts are injected as aliquots with a long needle horizontally (parallel to the surface) to avoid damaging structures outside the target area. The needle is inserted from several points and in variable directions and planes to achieve an even and natural appearing distribution of the graft.

[0022] When used purely for tissue quality improvement, the solved ASCs are injected in the dermis and subdermal with a thin sharp needle and evenly distributed in the target area. The incision and injection sites are sutured and postoperative compression garments are applied to the donor sites and in some cases also to the recipient sites.

Clinical benefits and novelty of the invention

[0023] ASC (ex-vivo expanded) enriched fat grafts or ASCs alone have never been used clinically for injection in the breast or in the face and has never been described in the literature nor has the inventor shared the idea of these clinical applications with others prior to the patent submission of US 61/839,578. The idea of enriching fat grafts with ex-vivo expanded ASCs in order to improve survival and quality of the fat grafts has been demonstrated in a murine model [3] and in a recent proof of concept study in humans [4], though as mentioned above the clinical applications of this invention has never been demonstrated (i.e. cosmetic breast filling and facial filling). It should be stressed that this invention (i.e. the application of ex-vivo expanded ASCs for the purpose of facial filling/rejuvenation and cosmetic breast filling/augmentation) is significantly different from the use of freshly isolated SVF, including a small fraction of non-expanded ASCs, for conventional so called "cell assisted lipo-filling". This method has been described in the literature and applied in humans with unpredictable clinical outcomes, not significantly better than conventional lipofilling [8]. The rationale for using ex-vivo expanded ASCs for facial filling is supported by the studies mentioned above, where it is demonstrated that stem cells survive after injection as opposed to fat cells. Additionally ASCs are very resistant to hypoxia and physical exposures [9-11]. By using stem cells alone as filling material a reliable residual volume/augmentation can be achieved.

[0024] There are many clinical benefits from making a biocompatible sustainable breast and facial filler, including natural appearance, non-immunogenicity, avoiding the side effects of artificial material and the procedure can be autologous. Most patients have natural fat reserves on the abdomen, thighs, arms and buttocks, which can be used. In this way the patients get a customized and desired body re-sculpturing. Autologous adipose tissue can easily be transplanted by simple liposuction and subsequent injection, with very little discomfort for the patients and with very little risks of side effects.

Examples

[0025] **Research Results - Proof of Concept Study** (analogous to the study design described in e.g. Kolle SF, Fischer-Nielsen A, Mathiasen AB, et al. Enrichment of autologous fat grafts with ex-vivo expanded adipose tissue-derived stem cells for graft survival: a randomised placebo-controlled trial. Lancet 2013; 382: 1113-20):

Aim of the study:

[0026] Fat grafts enriched with high dose autologous ex-vivo expanded adipose tissue-derived stem cells (ASCs) is compared to Non-enriched fat grafts (conventional fat grafting).

Study design:

[0027] Purified fat grafts, one with and one without ASC enrichment (control) are prepared for each participant. The fat grafts are injected subcutaneously.

[0028] A concentration of $20x10^6$ ASCs per mL enriched fat graft is chosen - approximately 2,000 times the physiological level.

[0029] The volumes of the injected fat grafts are measured by magnetic resonance imaging (MRI) immediately after injection and after 121 days and compared to the baseline MRI.

Result:

[0030] Compared with the control grafts, the ASC-enriched fat grafts have significantly higher residual volumes. No serious adverse events have been observed.

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[0031]

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PATENTKRAV

1. Fremgangsmåde til fremstilling af en sammensætning omfattende ex-vivo ekspanderede primærkultur (P0) stamceller stammende fra fedtvæv (ASC'er) blandet med høstet fedtvæv; hvilken fremgangsmåde omfatter:

- ex-vivo ekspandering af ASC'er fra en isoleret stromal vaskulær fraktion (SVF) i et vækstmedie bestående af Dulbecco's modificeret Eagle's medie (DMEM) eller alpha minimal essentiel medie (α-MEM),1-5% penicillin-streptomycin, 1-5 IU/ml konserveringsmiddelfri heparin og 2-20% pooled humant thrombocytlysat (pHPL),
- høstning af de nævnte ASC'er i en primær passage (P0), og
- blanding af de nævnte ASC'er med høstet fedtvæv i et blandingsforhold på 20 x 10^6 - 20 x 10^7 ASC'er/ml fedt.

2. Sammensætning omfattende ex-vivo ekspanderet primær kultur (P0) fra fedtvæv stammende stamceller (ASC'er) blandet med høstet fedtvæv i et blandingsforhold på i det mindste 20 x 10^6 - 20 x 10^7 ASC'er/ml fedt, hvor de nævnte ex-vivo ekspanderede primær kultur (P0) fra fedtvæv stammende stamceller (ASC'er) er dyrket i et vækstmedie bestående af Dulbecco's modificeret Eagle's medie (DMEM) eller alpha minimal essentiel medie (α-MEM),1-5% penicillin-streptomycin, 1-5 IU/ml konserveringsmiddelfri heparin og 2-20% pooled humant thrombocytlysat (pHPL) og som er høstet i den primære passage (P0).

3. Sammensætning ifølge krav 2 til anvendelse som et brystfyldningsmiddel, et ansigtsfyldningsmiddel, til kosmetisk brystfyldning, eller til kosmetisk ansigtsfyldning.

4. Kosmetisk fremgangsmåde til brystfyldning, hvor ex-vivo ekspanderede primær kultur (P0) fra fedtvæv stammende stamceller (ASC'er) blandes med høstet fedtvæv i et blandingsforhold på i det mindste 20 x 10^6 - 20 x 10^7 ASC'er/ml fedt, hvor de primære kultur (P0) fra fedtvæv stammende stamceller (ASC'er) er dyrket i et vækstmedie bestående af Dulbecco's modificeret Eagle's medie (DMEM) eller alpha minimal essentiel medie (α-MEM),1-5% penicillin-streptomycin, 1-5 IU/ml konserveringsmiddelfri heparin og 2-20% pooled humant thrombocytlysat (pHPL) og er høstet i den primære passage (P0), og hvor fedtet injiceres som alikvoter eller som strenge med en lang nål, horisontalt (parallelt med kroppen) ved indsætning af nålen fra adskillige punkter omkring brystvortekanten og ved adskillige punkter ved den under brystet

liggende fold, i forskellige retninger og planer for opnåelse af en jævn og naturligt udseende fordeling af transplantatet.

5. Kosmetisk fremgangsmåde til ansigtsfyldning, hvor ex-vivo ekspanderede primær kultur (P0) fra fedtvæv stammende stamceller (ASC'er) blandes med høstet fedtvæv i et blandingsforhold på i det mindste 20 x 10^6 - 20 x 10^7 ASC'er/ml fedt, hvor de primære kultur (P0) fra fedtvæv stammende stamceller (ASC'er) er dyrket i et vækstmedie bestående af Dulbecco's modificeret Eagle's medie (DMEM) eller alpha minimal essentiel medie (α-MEM),1-5% penicillin-streptomycin, 1-5 IU/ml konserveringsmiddelfri heparin og 2-20% pooled humant thrombocytlysat (pHPL) og er høstet i den primære passage (P0), og hvor fedttransplantat injiceres som alikvoter eller som strenge med en lang nål, horisontalt (parallelt med overfladen) for at undgå beskadigelse af strukturer udenfor målområdet, og nålen indsættes fra adskillige punkter og i forskellige retninger og planer for opnåelse af en jævn og naturligt udseende fordeling af transplantatet.

6. Kosmetisk fremgangsmåde til ansigtsfyldning, hvor ex-vivo ekspanderede primær kultur (P0) fra fedtvæv stammende stamceller (ASC'er) blandes med høstet fedtvæv i et blandingsforhold på i det mindste 20 x 10^6 - 20 x 10^7 ASC'er/ml fedt, hvor de primære kultur (P0) fra fedtvæv stammende stamceller (ASC'er) er dyrket i et vækstmedie bestående af Dulbecco's modificeret Eagle's medie (DMEM) eller alpha minimal essentiel medie (α-MEM),1-5% penicillin-streptomycin, 1-5 IU/ml konserveringsmiddelfri heparin og 2-20% pooled humant thrombocytlysat (pHPL) og er høstet i den primære passage (P0), og hvor ASC'er injiceres i dermis med en tynd skarp nål og jævnt fordelt i målområdet og indstiks- og injektionsstederne syes og post-operativ kompressionsbeklædning påføres donorpositionerne og i visse tilfælde også modtage positionerne.

7. Kosmetisk fremgangsmåde til indføring af et middel i huden, hvor midlet omfatter ex-vivo ekspanderede primær kultur (P0) fra fedtvæv stammende stamceller (ASC'er) blandet med høstet fedtvæv i et blandingsforhold på i det mindste 20 x 10^6 - 20 x 10^7 ASC'er/ml fedt, hvor de primære kultur (P0) fra fedtvæv stammende stamceller (ASC'er) er dyrket i et vækstmedie bestående af Dulbecco's modificeret Eagle's medie (DMEM) eller alpha minimal essentiel medie (α-MEM),1-5% penicillin-streptomycin, 1-5 IU/ml konserveringsmiddelfri heparin og 2-20% pooled humant thrombocytlysat (pHPL) og er høstet i den primære passage (P0).