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Development of Injectable Allograft Adipose Matrix for Soft Tissue Filling: From Conception to Clinical Trial

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BACKGROUND: There is a clinical need for an off-the-shelf bioinductive soft-tissue replacement in reconstructive surgery. Our group developed injectable Allograft Adipose Matrix (AAM) as a solution, derived from cadaveric human subcutaneous adipose tissue through a decellularization and milling process. The final form is lyophilized powder rehydrated before use. The aim was to demonstrate the translational development of AAM from conception to clinical study.

METHODS: In vitro and animal studies (using immunocompromised and wild type rodent models) were conducted to determine cellular ingrowth, vasculogenesis, adipogenesis and volume retention. A 16-week prospective clinical study evaluated subcutaneous AAM injection (2.5-5cc) of the dorsal wrist of 15 subjects to determine patient safety, graft retention, and histological characteristics. A clinical trial was then conducted by injecting 20cc of AAM into 6 individual abdominal subcutaneous sites of 10 subjects. Subjects were randomized to panniculectomy either 3 or 6 months after injection, and biopsies were taken at 1 and 2 months. Safety of AAM and histology of specimens obtained at biopsy and surgery were determined.

RESULTS: In vitro seeding of ASCs on AAM, showed attachment and proliferation of ASCs for 3 days, followed by production of new matrix within 7 days and changes to adipocyte morphology. AAM injected on the dorsum of immunocompromised nude mice supported adipogenesis at 6 weeks, with progressive increase in adipocyte frequency at 12–24 weeks and graft retention of 44±16% at 24 weeks. AAM injected in the dorsal flanks of immunocompetent Fisher rats showed higher graft retention (89±16%) up to 3 weeks, and induction of anti-inflammatory M2a macrophages as early as 72 hours compared to controls and alternative ECM derived products. The prospective clinical study evaluating dorsal wrist injections showed a graft retention of 47.14% at 16 weeks, with no histological evidence of inflammation or necrosis and no adverse events. The clinical trial demonstrated that larger volumes of AAM injections were tolerated well with no reactions. Clinical safety and graft retention were demonstrated at 6 months with histological evidence of adipogenesis and presence of endothelial cells. The only adverse event was surgical site infection in one out of 60 sites, which occurred after a biopsy.

CONCLUSION: AAM is a novel off-the-shelf adipose-derived injectable matrix, which represents a safe alternative for soft-tissue reconstruction. Bio-inductive AAM shows favorable volume retention, cellular infiltration, and de-novo adipogenesis from endogenous precursor cells.

The Immunophenotype of Adipocyte Stromal Vascular Cells Varies between Patients with Breast Cancer, Lipodystrophy and Macromastia

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BACKGROUND: Stromal vascular fraction (SVF) cells are embedded in adipose tissue and work synergistically when used in reconstructive and cosmetic breast procedures. SVF cells have been shown to promote tissue regeneration leading to improved wound healing and graft retention. SVF cells have become an attractive option for autologous applications in regenerative medicine due to easy access and processing. However, there is inherent
variability in the therapeutic potential of SVF cells, and the clinical outcomes vary from for any one patient to another. Therefore, this study investigated the regenerative and immunomodulatory properties of thirteen medically diverse human donors.

METHODS: Primary lipoaspirate samples were derived from either thigh, abdominal, or axillary fat pads. The donors represented medical histories corresponding to breast cancer (donors 1–6, at least one year in remission), lipodystrophy (donors 7–10), or macromastia (donors 11–13). SVF cells were isolated using established protocols and assessed for yield, self-renewal capacity, viability, differentiation potential, proliferation, and immunomodulatory activity.

RESULTS: Cell yield varied was on average 1–6 x 10^5 cells/ml lipoaspirate, and average post-thaw cell viability was 79% (median: 78%, range: 70–90%). The average self-renewal capacity of donor SVF cells was 3.6% (median: 3.6%, range: 1.6–7.0%). There was a strong trend towards increased clonogenic potential in breast cancer donors (p = 0.06). The differentiation potential for the osteogenic and adipogenic lineages in these samples was diminished (p > 0.05). Prior history of breast cancer did not appear to affect the immunomodulatory activity of donor SVF cells stimulated by pro-inflammatory conditions (p > 0.05).

However, SVF cells derived from patients with breast cancer in remission had a higher baseline expression of IL-6, IL-8, MCP-1, and IFN-γ.

CONCLUSION: Correlation analyses of therapeutic parameters across all donors identified positive correlations for the expression of pro-inflammatory cytokines interleukin IL6, IL8, and monocyte chemoattractant protein with each other. Samples obtained from patients in remission from breast cancer showed increased self-renewal as well as decreased differentiation potential and increased inflammatory cytokine production. These results could be relevant for determining clinical treatment strategies for patients with a previous breast cancer diagnosis. The significant donor-to-donor variability observed for the measured parameters supports the need for standardizing both the source of cells for therapeutic procedures as well as the assessments used to predict clinical outcomes.

The Impact of N-Acetylcysteine on Autologous Fat Graft - First-in-Human Pilot Study

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The aim of this pilot study was to determine whether N-acetylcysteine (NAC) administered at the fat graft donor site during the harvest procedure reduces oxidative stress and thus, improves graft survivability.

The study included 15 women with mean age of 31.8 years (range, 23–39 years). A 200-ml adipose tissue graft was harvested from each thigh in each study subject. The procedure of graft harvesting from one thigh, considered as the control (n=15), included infiltration with a standard tumescent fluid. During harvesting the graft from the other thigh, a tumescent fluid containing NAC (Sandoz, Holzkirchen, Germany) was used (NAC group, n=15). Plastic surgeon who conducted the procedure was blinded to the type of administered fluid. All patients were followed-up for 6-months for potential adverse events associated with administration of the modified tumescent fluid. A 65-ml adipose tissue sample from each graft was subjected to immediate and postponed biochemical analysis, flow cytometric assay and Quantitative Real-Time Reverse Transcription Polymerase Chain Reaction (qRT-PCR). The rest of the graft was used for correction of breast asymmetry, to determine the degree of fat resorption based on the comparison of pre- and postoperative MRI findings (separate study).

During biochemical tests, the severity of oxidative stress was determined based on the levels of reactive oxygen species (ROS) and nitric oxide (NO), along with the concentration and activity of superoxide dismutase (SOD). qRT-PCR analysis included targets linked with oxidative stress (GPX-3, hsCAT, hsSOD, iNOS, HO-1), angiogenesis (VEGF, ANG-2) and adipogenesis (PPAR-γ, C/EBP β).

Normal distribution of the study variables was verified with Kolmogorov-Smirnov test. Intragroup comparisons were carried out with Wilcoxon signed-rank test. All calculations were conducted with Statistica 10 (StatSoft, Tulsa, OK, USA), with the threshold of statistical significance set at p<0.05.