Is the Reparative Efficacy of Adipose-Derived Stem Cells Affected by Anatomical Harvest Site?

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Abstract

Lipomodelling is gaining favor to correct contour deformities after breast-conserving surgery for breast cancer. Some patients require repeated procedures to achieve the desired results. While the reasons for this may be multifactorial, we propose that the regenerative quality of stem cells within lipoaspirate may differ as a function of anatomical harvest site.

Patients: All patients undergoing lipomodelling at the Royal Liverpool Hospital were invited to take part in the study. Consent for the Liverpool Tissue Bank was obtained (ethical approval in place).

Methods: Fat was harvested using 10-mL syringes, 3-mmHg negative pressure, from 5 anatomical sites. Lipoaspirate was sent directly to the laboratory for processing. Stem cells were acquired from the samples using collagenase digestion and centrifugation before being subject to culture, and subsequent experimental interrogation investigating fundamental cellular properties (metabolic activity, protein production, proliferation), and stem cell characteristics (CD marker expression and differentiation).

Results: Since June 2015, 104 samples have been processed from 33 patients, with harvest locations typically including epigastrium, lower abdomen, flank, and inner and outer thighs. This has enabled us to optimize a hospital-to-laboratory workflow, and conclude a reliable and reproducible protocol for culturing viable cells from lipoaspirate tissue. Despite the relative infancy of this study, adipogenic differentiation confirmed using Oil Red O staining has suggested that cells from different anatomical origins within the same individual do vary in their plasticity. This phenomenon will be explored further and in considerably more depth throughout the remainder of this work.

Conclusions: In this preliminary work, stem cells derived from lipoaspirate appear to vary in their plasticity as a function of anatomical harvest site.

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Introduction

The history of fat grafting in breast surgery dates back to German surgeon Viktor Czerny (1842-1916), who first transplanted a lipoma to the breast following partial mastectomy. His German peer Erich Lexer (1867-1937) further developed a vast array of fat-grafting techniques in the field of maxillofacial surgery. He applied his techniques to surgery of the breast and hands. The usual place of fat harvest was the lateral thigh. The potential of fat grafting was further explored by Sir Harold Gilles (1882–1960), who used fat grafts to heal facial wounds in soldiers from World War I.[1]

Fat grafting gradually lost favor over the first half of the 20th century due to long-term results showing unpredictable fat resorption rates and a tendency to form fibrotic tissue or oily cysts. It was not until the advent of liposuction in the field of cosmetic surgery that fat injection regained popularity. Abundant lipoaspirate that was being discarded as waste represents a rich source of adipose-derived stem cells (ADSCs), and hence an interest in autologous fat transfer was reborn. It was Sydney Coleman who published his technique of fat harvest and re-injection, which attained the best results due to minimal trauma to the adipocytes, removal of the oily layer, and placement in multiple tunnels and in close contact with a well-vascularized bed.[2] The Coleman technique is still in use today.

Stem Cell Therapy

Techniques for isolating and processing lipoaspirate to yield ADSCs that are suitable for use as a component of a cell-based regenerative therapy has been well described.[3-6] The stem cells themselves hold the regenerative and reparative properties that make them so attractive for clinical application.[1,7]

In 2007, Rigotti et al[8] published results of their clinical pilot study using lipoaspirate to promote healing in the context of radiation-induced injury after breast cancer treatment. This study included 20 consecutive patients with LENT-SOMA grade 3 (severe symptoms) or grade 4 (irreversible functional damage) symptoms. The authors observed significant improvement in symptoms in all but 1 patient after fat injection, and used ultrastructural analyses to relate this to the growth of a microvascular bed, which promotes adipocyte viability following fat injection.

The work of Rigotti et al is merely one example that showed fat

injection to promote tissue repair and regeneration. Adipose transfer and the use of ADSCs has similar applications in burns and complex wound management.[9] With this consistently increasing list of soft-tissue defects in which it is showing promise, it is highly likely that the full potential of fat grafting is yet to be appreciated.

Current Practice

The process of using lipoaspirate harvested from liposuction for autologous fat transfer is now relatively well established. In the field of breast surgery, this has traditionally been used to correct deformities after breast-conserving surgery. This is commonly referred to as "lipomodelling."[3,10]

Lipomodelling has been performed without regulation until recently, when the Association of Breast Surgeons (ABS), the British Association of Plastic Reconstructive and Aesthetic Surgeons (BAPRAS), and the British Association of Aesthetic Plastic Surgeons (BAAPS) released their joint clinical guidelines in 2012.[11] The objective of these guidelines is to "support training and audit, inform appropriate use and promote safety." The joint guidelines were quickly followed by an *Interventional Procedure Guidance* (IPG417) published by the National Institute for Health and Care Excellence (NICE).[12]

There is a need for further research to be conducted in order to further understand, improve, and personalize the clinical outcomes and long-term safety of the technique.

Lipomodelling is gaining favor among the surgical community, and it appears to be well tolerated by patients with a good safety profile. However, there is variability in the success rate seen in patients who undergo this procedure.[10] It is accepted that a degree of fat resorption will occur after fat transfer, necessitating repeat procedures. This is usually clinically evident due to volume loss at around 4 to 6 months post-procedure, with the extent of resorption varying between patients that may be related to factors such as patient comorbidities, including obesity, diabetes mellitus, or age.[12] Another theory is that the quality of the lipoaspirate differs depending on the anatomical harvest location.[14]There are limited published data that attempt to address these questions that we hypothesize have a significant impact on the efficacy of any lipomodelling procedure.

To investigate this hypothesis with the objective of adding both clinical and scientific impact to the current understanding of the impact of stem cell quality in fat-based regenerative medicine, we have set up a study in association with the Royal Liverpool University Hospitals Trust, The University of Liverpool, and Liverpool John Moores University.

Methods

All female patients undergoing lipomodelling under the care of 2 named oncoplastic breast surgeons are invited, without obligation, to take part in the study. Consent is obtained from the Liverpool Tissue Bank (http://www.liv.ac.uk/ltb), which is an established biorepository with ethical approval in place. Samples are obtained in theatre using the Coleman technique. Fresh lipoaspirate from varying anatomical sites is sent to the laboratory for analysis.

Tissue Culture

Adipose Digestion Protocol

Our initial work in this area has partly focused on assessing whether we are preparing our cells for delivery in the most appropriate manner in theatre, using differential centrifugation to remove nonadipose material. To this end, we have modified a standard adipose stromal vascular fraction isolation protocol to yield 3 culture fractions for experimental interrogation.

Explanted tissue was transferred into a 30-mL tube, adjusted to a volume of 30 mL using media 199 without serum, and centrifuged at 500G for 3 minutes. Floating fat tissue was collected from the top of the tubes using forceps (adipose) or a pipette (lipoaspirate), and transferred to a clean 30-mL universal tube and weighed. The remaining cell pellet containing mostly hematologic cells was re-suspended in 5-mL media 199 with 10% FCS, 1% penicillin/streptomycin, and seeded into a single well of a 6-well plate. **These samples were designated Fraction A.**

Remaining adipose tissue was made up to a volume of 10 mL using media 199 without serum with 100 µL of 1g/mL collagenase rolled for 30 minutes at 37°C. After this time has elapsed, samples were spun at 500G for 10 minutes. Floating undigested fat was collected from the top of tube using a pipette and added to a T25 flask containing 5-mL media 199 with 10% FCS, 1% penicillin/streptomycin. **These samples were designated Fraction C.**

The remaining supernatant was discarded, and the cell pellet then re-suspended in 5-mL media 199 with 10% FCS, 1% Ab, and added to a single well of a 6-well plate. **These samples were designated Fraction B.**

Experimentation

Cells are cultured until confluence before being taken forward into an experimental workflow, which has been designed to assess both donor- and harvest site specific differences in both the fundamental cellular properties of the cells and to more targeted investigations towards assessing their stem cell characteristics.

Fundamentally, cells are investigated for their metabolic activity using the Resazurin assay, numeration/proliferation using the CyQUANT assay (ThermoFisher Scientific, UK), and protein production using the Bradford assay (Sigma-Aldrich, UK). To interrogate the stem cell specific properties of the cells, they are subjected to differentiation assays, with a particular focus on adipogenic differentiation. More recently, cells are also subjected to flow cytometric analysis using a gold standard adult stem cell characterization panel supplied by BD Biosciences that considers the markers CD44, CD90, CD105, and CD117.

One hundred-and-three samples from 31 patients have been throughout the laboratory for the initial collection, logistics,

culture, and assay optimization phase of this study. Typically, for each patient there have been between 3 and 5 anatomical sites that frequently include upper abdomen (epigastrium), lower abdomen, flank, and inner thigh and outer thighs.

We have carried out adipogenic differentiation studies on 22 samples from 5 patients using both B and C Fractions from 3 to 5 anatomical sites. We have shown that these cells can be driven down an adipogenic lineage, confirming plasticity towards a cell type pivotal to their success in their ultimate regenerative application. Adipogenic differentiation has been confirmed using Oil Red O staining for lipids and was measured at 3 time points: 7, 14, and 28 days.

Although patient numbers are small at present, early results from this differentiation work appear to indicate that a heterogeneity exists in the differentiation potential of these cells as a specific function of their harvest location. This early positive work is promising towards supporting our hypothesis that diversity in the potential outcome of this technique is largely driven by variation in the regenerative capacity of the stem cells within a patient's adipose tissue.

Interestingly, we have also attempted osteogenic differentiation, confirmed using von Kossa staining mineralization, which although only performed on 1 patient to date, also supported the trend observed with the adipogenic differentiation work.

Conclusions

A technique that enabled the isolation and culture of ADSCs from human lipoaspirate tissue has been established. It has been shown that the derived cells can be pushed and driven towards both adipogenic and osteogenic differentiation lineages.

Most significant, but certainly requiring repetition, was the finding that a heterogeneity appears to exist in the differentiation capacity of cells from different harvest locations, which will be explored further and substantially throughout the lifetime of this study.

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