Philadelphia College of Osteopathic Medicine

Annual Progress Report: 2010 Formula Grant

Reporting Period

January 1, 2011 – June 30, 2011

Formula Grant Overview

The Philadelphia College of Osteopathic Medicine received $19,326 in formula funds for the grant award period January 1, 2011 through December 31, 2014. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Effect of Liposuction Technique on the Viability and Differentiation of Adipose Derived Stem Cells - Tissue engineering and regenerative medicine is a rapidly developing field of research in which new materials and strategies are proposed for the repair of lost or degenerative tissues. A key factor in these strategies is a ready supply of cells to populate these replacement materials. Adipose derived stem cells have been suggested as a source of autologous cells for potential use in numerous therapies. However, while the use of liposuction techniques for the recovery of these cells from adipose tissue has been proposed, to date, no work has shown the desirability of one potential technique over another. We propose to determine the role of liposuction method on the ability to isolate stem cells from fat for tissue engineering purposes. The results of this work should guide reconstructive strategies in the future, significantly impacting the standard of care.

Anticipated Duration of the Project

1/1/2011 – 12/31/2014

Project Overview

The primary objective of the project is to determine the role of liposuction method on the ability to isolate stem cells from fat for tissue engineering purposes. We will specifically test the hypothesis that the method by which adipose tissue is recovered during liposuction will impact both the quality and differentiation potential of adipose-derived stem cells.

In Specific Aim 1, we will determine the role liposuction technique plays in altering the viability of retrieved stem cells from adipose tissue. Our clinical collaborator will be providing us with material recovered from liposuction procedures. These procedures will utilize one of four different devices. We will examine material from patients treated with SmartlipoMPX, a brand of laser lipolysis; VASER liposelection, a brand of ultrasonic liposuction; the Custom Acoustic Liposuction device, a powered liposuction cannula; and the Body-Jet Liposuction device, a water-jet assisted liposuction device. These devices will be used in isolation and in combination.

Cells will be isolated from recovered material and analyzed for both viability and concentration of stem cells. Viability of isolated cells will be evaluated immediately and normalized by material volume. Percentages of live and dead cells will be evaluated by flow cytometry. The percentage of stem cells among the isolated cells will be determined by examining the expression of three different cell surface markers, CD31, CD34 and CD45, again by flow cytometry. Adipose derived stem cells express CD34, but are negative for CD31 and CD45. In Specific Aim 2, we will confirm the potential fates of adipose-derived stem cells as a function of liposuction technique. Isolated cells will be cultured in fate specific media. Potential of cells to differentiate into one of three different fates will be evaluated. Osteoblastic, chondrocytic and adipocytic cell fates have been chosen as endpoints and will be evaluated using differentiation markers for each cell type. Accumulated data will allow us to compare each technique individually and in combination on the ability to recover stem cells from the aspirated material. In addition, the role of anatomical position, repeated procedures and anesthetic composition on the viability and differentiation potential of recovered stem cells will be examined.

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Other Participating Researchers and Employers

Christopher S. Adams, PhD - employed by Philadelphia College of Osteopathic Medicine
Victoria Falcone, DO - employed by Physician Body Solutions

Expected Research Outcomes and Benefits

Much of the current work in the field of tissue engineering and regenerative medicine is focused on the development of material to replace those lost through trauma and aging. In the field of orthopaedics, for example, these approaches have addressed the problem of wholesale bone loss associated with tumor resection or traumatic damage. A primary problem with such strategies is populating the replacement material with viable cells. Replacement by locally derived repair cells is limited by both the supply of such cells and their ability to penetrate the material. Seeding of the material with cells prior to implantation resolves many of those issues, but raises an even more complex question concerning what could be the source of these cells. While donor cells collected from other patients may one day prove to be an adequate strategy, the current standard of care is the use of autologous cells from the patients themselves. With the identification of stem cells in liposuction aspirate, called adipose derived stem cells, a ready supply of these cells appears to have been identified. Therefore, if, in the future, liposuction is undertaken as a first step in a repair scenario, the question arises, what is the ideal method of harvesting adipose tissue, so as to maximize the recovery of adipose derived stem cells? The focus of this project is toward answering that question. We expect that, with the completion of this project, we will be able to advise how to best maximize the recovery of such cells.

Summary of Research Completed

In the first six months of this study (January 2011-June 2011), we optimized the fat and stem cell isolation procedure following clinical aspiration with different liposuction methods. We examined four forms of liposuction: powered liposuction (Custom Acoustic), water-jet assisted liposuction (Body-Jet), ultrasonic liposuction (VASER) and laser lipolysis (SmartLipoMPX), both alone and in combination with each other. The technique utilized by the physician collecting the sample depended on a number of factors including personal preference, patient comfort, availability of the technique, ease of disruption of the adipose tissue, and body location that underwent the procedure. All of these factors can vary according to patient tolerance and physician preference, and therefore it was imperative to this study to hold the operating physician, our collaborator, Victoria Falcone, DO, as a constant parameter to decrease these variables. A total of 40 patients were included in this study so far with an age range of 15 to 72 years. The greatest number of patients fell in the 30-39 years of age group. The patient population analyzed for this study included both sexes with a majority of samples collected from the abdomen in patients that had not had a prior surgical treatment in that area.

Optimization of the stem cell isolation procedure protocol demonstrated that the number of viable cells lost during the isolation process is negligible regardless of the lipoaspirate collection method (Figure 1). Figure 1 shows the infranatant after each of the wash steps during the isolation procedure was collected and analyzed with Guava® Viacount® Reagent. None of the infranatants exceeded 13% of the total number of cells in the final cell isolates compared with final cell concentrations of 1000 events performed on the Guava EasyCyte Plus system cell sorter.

Cell viability data was also collected to compare samples immediately after isolation (Fresh) or following para-formaldehyde fixative addition and sample storage at 4°C for a minimum of 24 hours (Fresh-Fixed). There are no significant differences between freshly isolated and counted preparations versus paraformaldehyde-fixed samples (Figure 2). For example, fixation of the Body Jet lipoaspirate samples did not appear to have much, if any effect on the number viable cells recovered. It did appear to increase the total number of cells, although none of the differences observed were found to be statistically significant.

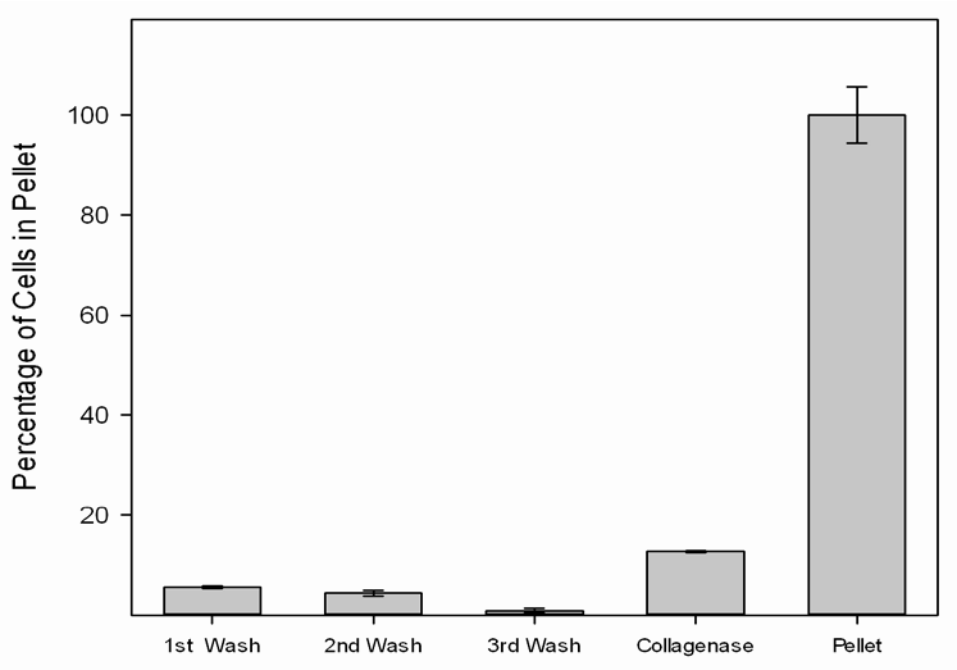

Figure 1. Percentages of Viable Cells Lost in Isolation Procedure Washes. Infranatant was subjected to cell count with Guava® Viacount® Reagent at each wash step of the isolation procedure. Percentage cells compared to the total cell number from the isolations (Pellet) were calculated and expressed for each infranatant.

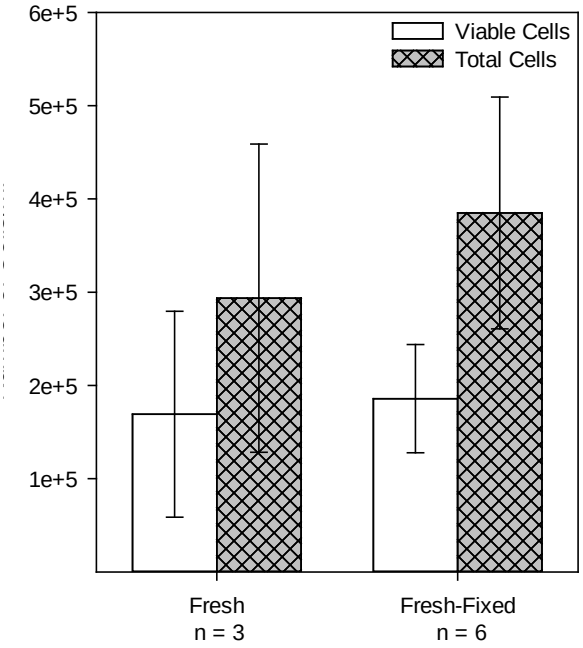

Figure 2. Cell Viability in Fresh Isolate Versus Fresh-Fixed Isolates. Differences in viable cells and total cell numbers per milliliter of isolate are represented here. Lipoaspirate from BodyJet technique were isolated with the optimized protocol and viability measured with Guava® Viacount® Reagent.