January 16, 2012

To Whom It May Concern:

Please find below the information requested for a final report regarding our ASERF Interim Research Grant funded study. Thank you kindly.

Sincerely,

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Your ASPS ID: 6550

Project Title: Skin regenerative potential of adipose-derived stem cells (ADSCs): in vivo nude mouse model

Abstract:

Purpose/Objectives:
Subjective skin improvements been reported after soft tissue augmentation of the face using autologous fat. Adipose-derived stem cells (ADSCs) have been implicated (i.e. via growth factor production). ADSCs have not yet, however, been shown to differentiate towards skin cell lineages. We suggest that direct in vivo evidence of ADSC differentiation towards skin cell lineages would further support their use as a skin regenerative treatment modality, expanding their application towards treatment of a variety of dermatologic pathologies. We aimed to provide in vivo evidence of ADSC dermal migration, and differentiation towards skin cell lineages, after subdermal fat grafting.

Methods:
Twelve male, GFP (green fluorescent protein) producing mice served as adipose tissue donors. Twenty-four nude mice served as recipients. Recipients were subdivided into four arms (6 mice/each arm). Experimental arms included nude mice that received whole inguinal adipose specimen (unrefined fat + ADSCs) (Group 1), ADSCs alone (Group 2), 1ml of refined adipose specimen + ADSCs (Group 3), or 1ml of refined adipose specimen without ADSCs (Group 4) engrafted, respectively, into the left parascapular subdermal plane. The right parascapular subdermal plane was subjected to one of two control parameters (1ml of phosphate buffered saline or sham surgery). Tissue was harvested at 8 weeks, sectioned, and subjected to confocal microscopy for identification of GFP producing ADSCs within the overlying skin. We anticipated co-localization of GFP with p63, an epidermal cell marker used to demonstrate ADSC differentiation towards epidermal cell lineages. Reverse transcriptase polymerase chain reaction (real time RT-PCR) was used for quantification of p63 expression among the experimental groups (n=6 each group). The statistical significance of the difference between group mean values was evaluated using the Student's t-test. *$p<0.05$; ** $p<0.01$

Findings/Results:

At tissue harvest, whole fat tissue specimens (Group 1) were noted to have subjectively increased blood vessel formation overlying engrafted specimens, suggestively supporting the known contribution ADSCs make towards neovascularization (Figure 1). Confocal microscopy of Group 1 (Figure 2) and Group 2 (Figure 3a) sections demonstrated ADSC cell migration into overlying dermal architecture. P63 co-localized to the GFP producing donor cells seen migrating through the dermis of recipient skin specimens (Figure 3b). Statistical analysis of RT-PCR for p63 demonstrated significantly increased levels in the refined fat + ADSC experimental group (Group 3), when compared to groups 1 and 4 (Figure 4). Group 2 was not included in this analysis as its ADSC cell population had been expanded in vitro, prior to implantation, which would have confounded the comparison.

Conclusions:

This is the first study to offer direct evidence of ADSC migration into the overlying skin architecture, suggesting ADSC cellular contribution to the overlying skin, after fat grafting, beyond paracrine mechanisms alone. Additionally we have offered the first account of direct differentiation of ADSCs towards epithelial cell lineages in vivo. As cell or tissue-based transplantation therapies for skin tissue injury are developed, these findings may have important implications in better understanding stem cell biology, and specifically may justify the treatment for pathologic skin conditions (i.e. systemic sclerosis).

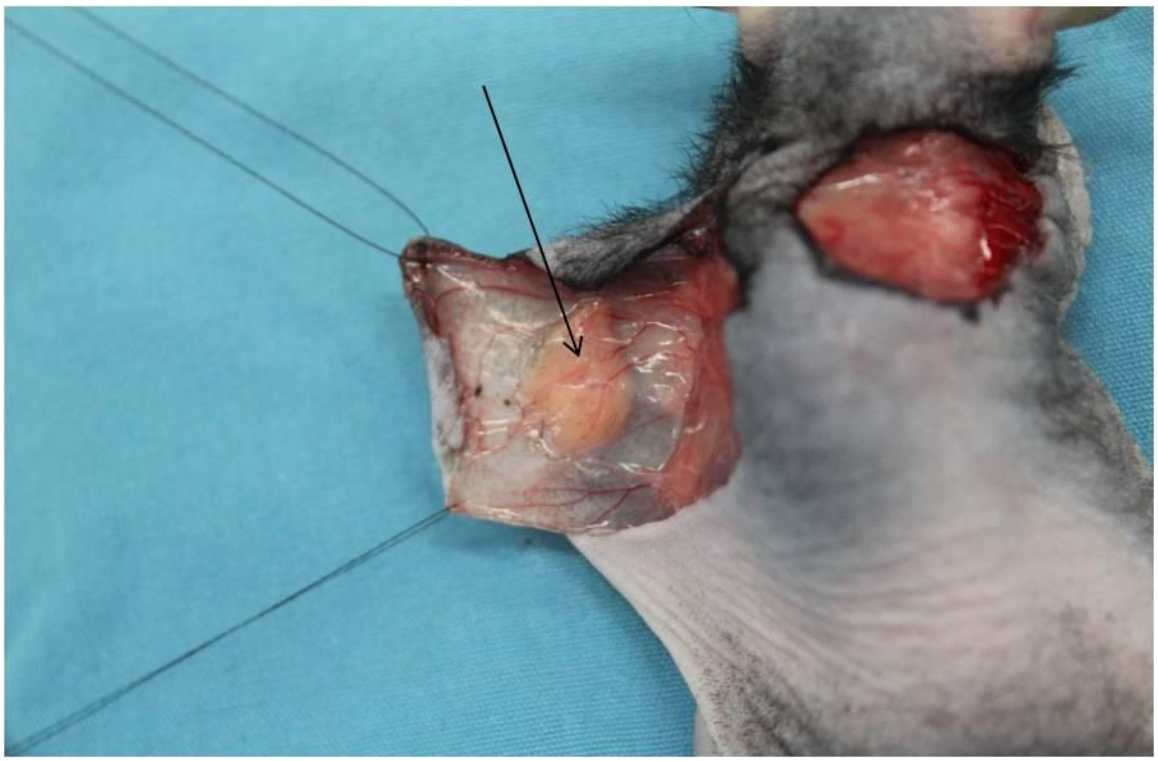

Figure 1: Left parascapular skin harvest from whole fat nude mouse recipient. Arrow denotes one of vessels feeding the engrafted specimen.

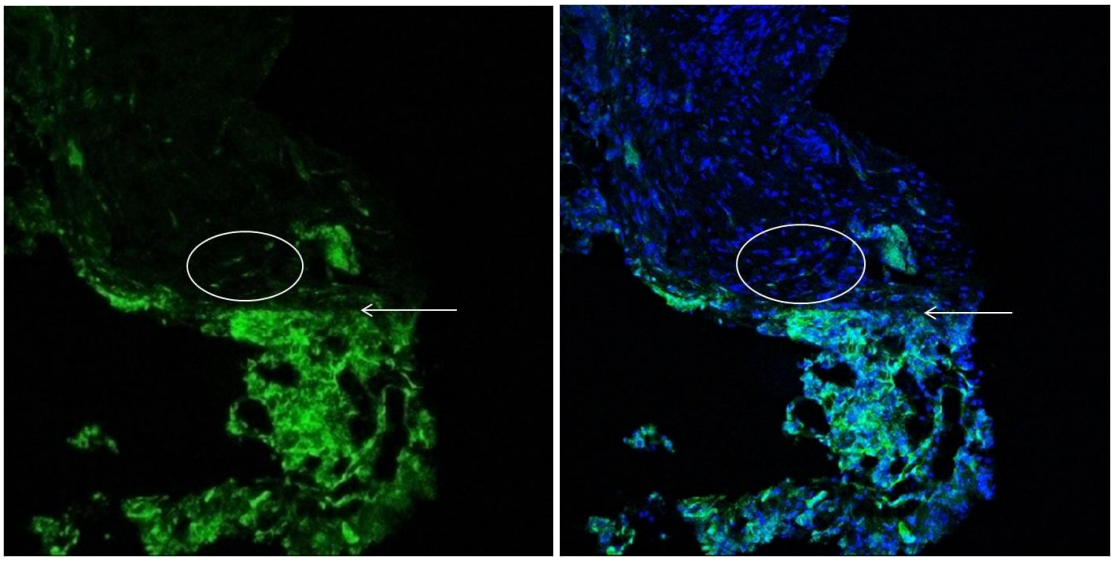

Figure 2: Tracking donor GFP producing cells after whole fat engraftment (Group 1). Confocal microscopy of dermal-subcutaneous tissue junction (arrows) of left parascapular skin specimen. Circles surround GFP fluorescent cells migrating into dermal tissue. Left – GFP producing cells. Right – merged cell nuclei (blue) with GFP fluorescence (green). Original magnification: X100

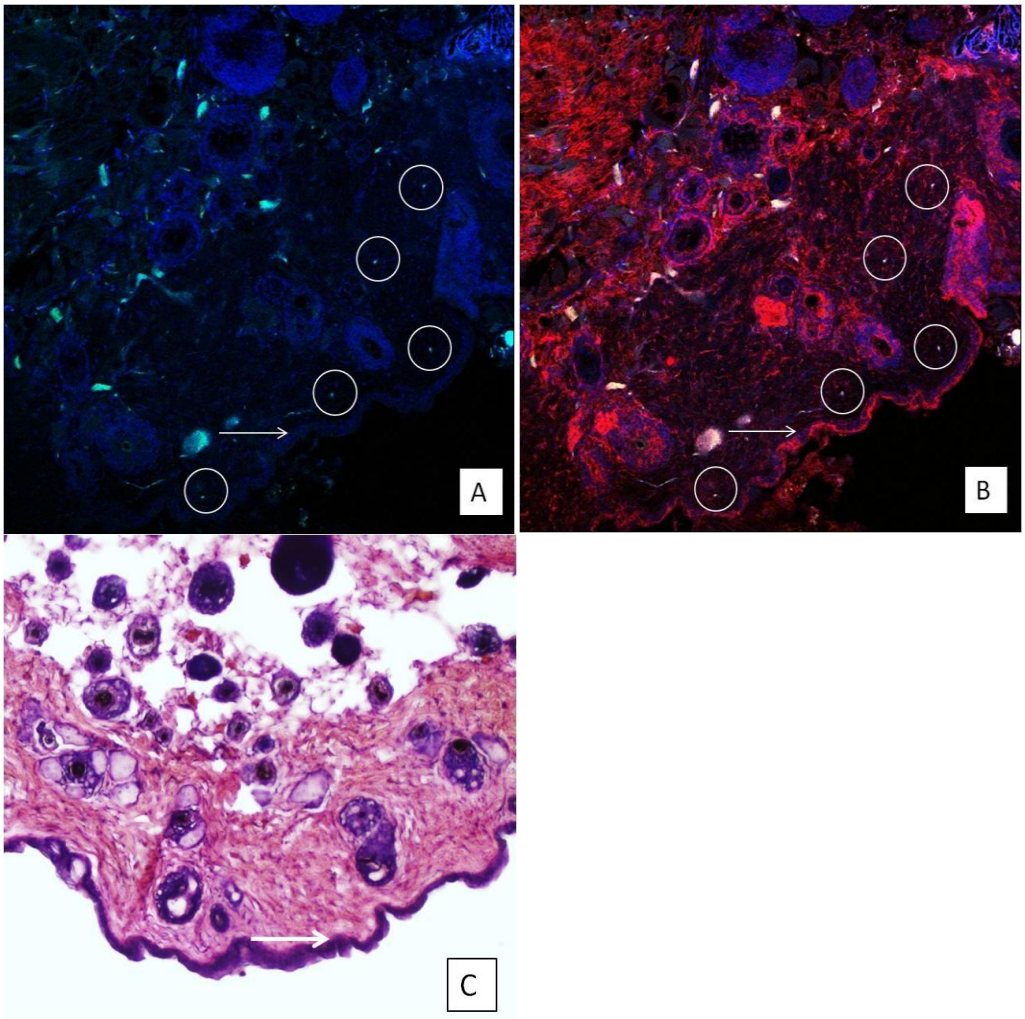

Figure 3: Epithelial cell lineage differentiation potential of engrafted ADSCs (Group 2). Confocal microscopy of skin specimens (A,B), after 8 weeks of in vivo engraftment. Image C is included for histologic orientation (H&E staining). Arrows define epidermal-dermal junction. Circles surround GFP producing cells. Image A – merged image of cell nuclei (blue) and GFP producing cells (green). Note cells co-localization to the dermis, indicating dermal migration of ADSCs. Image B – merged image of nuclei (blue), and p63 epithelial cell surface marker (red). Note pink co-localization of p63 with the nuclei of the ADSCs found within the dermis, indicating cellular differentiation of ADSCs to epithelial cell lineages. Original magnification: X200 (A, B, C).

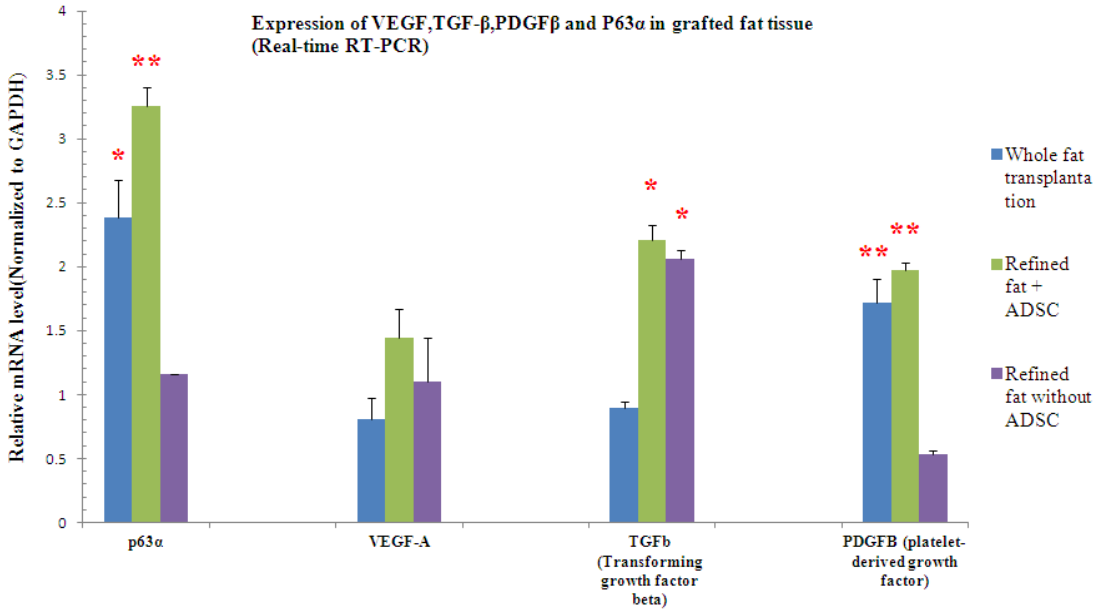

Figure 4: Contribution of ADSCs to skin cell lineages and paracrine effect after fat engraftment. Real-time polymerase chain reaction (RT-PCR) analysis of epidermal cell marker P63 and paracrine effect (VEGF and TGFβ) in engrafted tissue from whole fat transplantation, refined fat+ADSC and refined fat without ADSC donor group. Gene expression of p63 and PDGFβ significantly increased in the refined fat + ADSC experimental group and whole fat transplantation group, when compared to refined fat without ADSC group. Gene expression of TGF-β significantly increased in both the refined fat + ADSC group and refined fat without ADSC group, when compared to the whole fat transplantation group. VEGF expression was not significantly different among all three groups. * $p<0.05$; ** $p<0.01$

Presentation(s):

Title: Skin regenerative potential of ADSCs in an in vivo nude mouse model
Organization: American Society for Aesthetic Plastic Surgery
Meeting: The Aesthetic Meeting 2012
Location: Vancouver, BC
Date: May 3-8, 2012 (Abstract Submitted - slated to present at research luncheon)

Publication(s):

Submission to Aesthetic Surgery Journal (ASJ) near complete.

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