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Cell-Assisted Lipotransfer Improves Volume Retention in Irradiated Recipient Sites and Rescues Radiation-Induced Skin Changes

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Abstract

Radiation therapy is not only a mainstay in the treatment of many malignancies but also results in collateral obliteration of microvasculature and dermal/subcutaneous fibrosis. Soft tissue reconstruction of hypovascular, irradiated recipient sites through fat grafting remains challenging; however, a coincident improvement in surrounding skin quality has been noted. Cell-assisted lipotransfer (CAL), the enrichment of fat with additional adipose-derived stem cells (ASCs) from the stromal vascular fraction, has been shown to improve fat volume retention, and enhanced outcomes may also be achieved with CAL at irradiated sites. Supplementing fat grafts with additional ASCs may also augment the regenerative effect on radiation-damaged skin. In this study, we demonstrate the ability for CAL to enhance fat graft volume retention when placed beneath the irradiated scalps of immunocompromised mice. Histologic metrics of fat graft survival were also appreciated, with improved structural qualities and vascularity. Finally, rehabilitation of radiation-induced soft tissue changes were also noted, as enhanced amelioration of dermal thickness, collagen content, skin vascularity, and biomechanical measures were all observed with CAL compared to unsupplemented fat grafts. Supplementation of fat grafts with ASCs therefore shows promise for reconstruction of complex soft tissue defects following adjuvant radiotherapy.

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Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

Author Contributions

A.L.: study design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, and final approval of manuscript; D.D. and A.J.W.: collection and/or assembly of data, data analysis and interpretation, and final approval of manuscript; K.J.P., E.R.Z., E.A.B., D.A.A., and M.S.H.: collection and/or assembly of data and final approval of manuscript; G.K.L. and G.C.G.: financial support, administrative support, and final approval of manuscript; M.T.L.: financial support, administrative support, provision of study material, and final approval of manuscript; D.C.W.: conception and design of study, financial support, administrative support, provision of study material, data analysis and interpretation, manuscript writing, and final approval of manuscript

Keywords

Cell-assisted lipotransfer; Irradiation; Radiation fibrosis; Fat grafting; Adipose-derived stromal cells; Adipose-derived stem cells

Introduction

Adjuvant radiation therapy is highly effective in reducing local recurrence risk following resection of various tumors [1, 2]. However, detrimental collateral changes frequently occur to the surrounding tissue, with macroscopic discoloration and loss of skin elasticity as well as microscopic obliteration of small vessels and dermal thickening, making reconstruction of irradiated wounds and soft-tissue defects challenging [3–5]. Rehabilitation may be addressed through autologous fat grafting, but transfer of avascular fat to hypovascular postradiation recipient beds has been associated with poor fat graft survival [6, 7]. Nonetheless, clinical fat grafting has been recognized to possess a regenerative effect, with improved skin quality and restoration of vascular networks and reduction in collagen disorganization [6]. Several lines of evidence support the notion that adipose-derived stem/stromal cells (ASCs) found within grafted fat confer much of this capacity. This includes reduction of fibrosis following mesenchymal cell injection and the known ability for ASCs to secrete both angiogenic and antiapoptotic growth factors [8–10]. Furthermore, enrichment of lipoaspirated fat with supplemental ASCs, known as cell-assisted lipotransfer (CAL), has been shown to enhance fat graft retention at healthy recipient sites [11, 12]. In this study, we determined the ability for CAL to promote fat graft survival in irradiated soft tissue and evaluated the capacity for supplemental ASCs to augment the regenerative effects of fat on the overlying fibrotic skin.

Materials and Methods

Animal Irradiation and Fat Grafting

All studies were performed in accordance with Stanford University animal guidelines. A total of 30 Gy external beam radiation was delivered to the scalp of adult 60-day-old male Crl:NU-*Foxn1*tm immunocompromised mice in six fractionated doses of 5 Gy each over 12 days, followed by 5 weeks of recovery [7]. Lipoaspirate was obtained from two healthy female donors, ages 45 and 49 years. ASCs were harvested from half of the lipoaspirate while the other half was processed for grafting by centrifugation [13, 14]. For CAL, 250,000 supplemental cells were added in a small volume (20 μ l) to a larger volume of fat (5 ml). Fat (200 μ l), with or without supplemental cells (10,000 cells per graft), was then injected beneath the scalp of age-matched irradiated and non-irradiated mice [7, 14–16]. This well-established mouse model facilitates in vivo study of human grafts with minimal immunological reaction.

Fat Graft Analysis

Fat graft volume retention was measured over 8 weeks using microcomputed tomography ($n = 6$ animals per group), and images were reconstructed as three-dimensional surfaces through cubic-spline interpolation [15]. Fat grafts were removed after 8 weeks, and eight hematoxylin and eosin (H&E) stained sections from each group were evaluated for graft

integrity, cysts/vacuoles, inflammation, and fibrosis by three blinded, independent reviewers [17]. CD31 fluorescent immunohistochemical staining (Ab28364; Abcam, Cambridge, MA, <http://www.abcam.com>) was performed to assess vascularity and then quantified by ImageJ analysis [16].

Skin Analysis

Scalp skin was harvested prior to and 8 weeks following grafting. Sections were stained with H&E and Masson's Trichrome to analyze dermal thickness and collagen density/structure. Vascularity was determined with CD31 immunohistochemical staining. Mechanical analysis of harvested skin was performed on a MTS Bionix 200 (MTS Systems, Eden Prairie, MN) with an Interface SM-10 force transducer. Stress-strain curves were generated to calculate Young's modulus.

Statistical Analysis

Data are presented as means \pm SE. Analyses of variance were used for multiple group comparisons, and two-tailed Student's *t* tests were used for comparisons between two groups. All analyses were performed using GraphPad Prism (GraphPad Software, La Jolla, CA). A *p* value $<.05$ was considered statistically significant.

Results

Volumetric analysis of fat grafts demonstrated significantly improved retention with CAL in irradiated scalps (Fig. 1A). Fat graft survival for CAL in irradiated animals ($76.2\% \pm 3.3\%$ at week 8) was similar to non-irradiated animals receiving CAL ($78.2\% \pm 2.6\%$) (Fig. 1B). Both groups outperformed fat alone in non-irradiated scalps ($68.8\% \pm 3.2\%$), and the worst volume retention was observed with unsupplemented fat grafts placed at irradiated recipient sites ($58.2\% \pm 3.6\%$). These findings are congruent with histological analyses which found the worst graft integrity and the greatest presence of cysts/vacuoles when fat alone was grafted into irradiated scalp (Fig. 2A). Both of these measures were significantly improved with CAL in irradiated animals. Increased graft vascularity, as shown by CD31 immunofluorescent staining, may have contributed to these histological findings. Not surprisingly, fat grafts in irradiated scalps had the lowest vascularity, and this was significantly enhanced by CAL (Fig. 2B). The ability for supplemental ASCs to secrete angiogenic cytokines when grafted with fat further support this observation [16].

The regenerative effect of fat on the overlying fibrotic skin was also enhanced by CAL. Radiation-induced dermal fibrosis was reflected in a thicker dermis ($215 \pm 20 \mu\text{m}$) and increased collagen content at baseline, and while fat grafts alone attenuated some of this pathologic change ($186 \pm 10 \mu\text{m}$), CAL was found to significantly reduce dermal thickness ($125 \pm 6 \mu\text{m}$) and collagen content, with amounts similar to non-irradiated skin ($121 \pm 6 \mu\text{m}$) 2 weeks after graft placement (Fig. 3A, 3B). Skin hypovascularity following radiation was also partially ameliorated by unsupplemented fat, as previously demonstrated, but was significantly improved with CAL (Fig. 3C) [7]. Parallel observations were made at 8 weeks, with notable improvement in dermal thickness, collagen content, and hypovascularity of irradiated skin overlying fat grafts without ASC supplementation. These observations,

however, were still greater with CAL (Fig. 3D–3F). From our results and others in which multiple points were sampled [7], these measurements did not vary significantly across skin overlying graft sites. Studies have shown persistent dysregulation of fibroinflammatory cytokines following radiation exposure, and fat grafting may reverse some of pathologic changes through downregulation of profibrotic factors such as transforming growth factor-beta [7, 18–20]. Further inhibition of Smad3 signaling in concert with potential immunomodulatory effects of supplemental ASCs in CAL may limit dermal collagen production, as observed in other settings [21–24].

These improved histologic findings in irradiated skin were also reflected in enhanced biomechanical metrics, as demonstrated by measures of material stiffness. Tensile testing generated stress-strain curves to determine the Young's modulus (Fig. 4A). Stiffness of irradiated skin 2 weeks after grafting decreased with unsupplemented fat, but this was further improved with CAL (Fig. 4B). By 8 weeks, CAL significantly reduced irradiated skin stiffness (13.0 ± 2.4 MPa, from 21.9 ± 2.4 MPa), returning measured levels to non-irradiated skin controls (14.3 ± 1.1 MPa) (Fig. 4C). These findings are congruent with previous studies showing improved elasticity in irradiated wounds injected with ASCs [25]. The advantages of CAL over fat grafting alone thus extend beyond histopathological improvements to provide a functional regenerative benefit, with supplemental ASCs augmenting the ability for fat to reverse many chronic radiation-induced cutaneous changes.

Discussion

Radiation therapy results in deleterious soft tissue changes which complicate soft tissue reconstruction. Fat graft outcomes, while worse at irradiated sites, can be significantly improved with ASC supplementation. Furthermore, CAL augments the regenerative effects of fat on the overlying skin, resulting in histopathologic and functional improvements of radiation-injured skin. Importantly, concern has been raised regarding use of stromal cells in the setting of prior malignancy, and while clinical studies such as RESTORE II have not demonstrated increased rates of cancer recurrence, need for long-term follow-up has thus far limited widespread clinical adoption. Nonetheless, CAL shows promise for reconstruction of soft tissue deficits following adjuvant radiotherapy, functioning both as an autologous filler and as a regenerative therapeutic.

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Significance Statement

Radiation therapy results in deleterious soft tissue changes which complicate soft tissue reconstruction. Fat graft outcomes, while worse at irradiated sites, can be significantly improved with adipose-derived stem cell supplementation, also known as cell-assisted lipotransfer. Furthermore, cell-assisted lipotransfer augments the regenerative effects of fat on the overlying skin, resulting in histopathologic and functional improvements of radiation-injured skin. Cell-assisted lipotransfer therefore shows promise for reconstruction of soft tissue deficits following adjuvant radiotherapy, functioning both as an autologous filler and as a regenerative therapeutic.

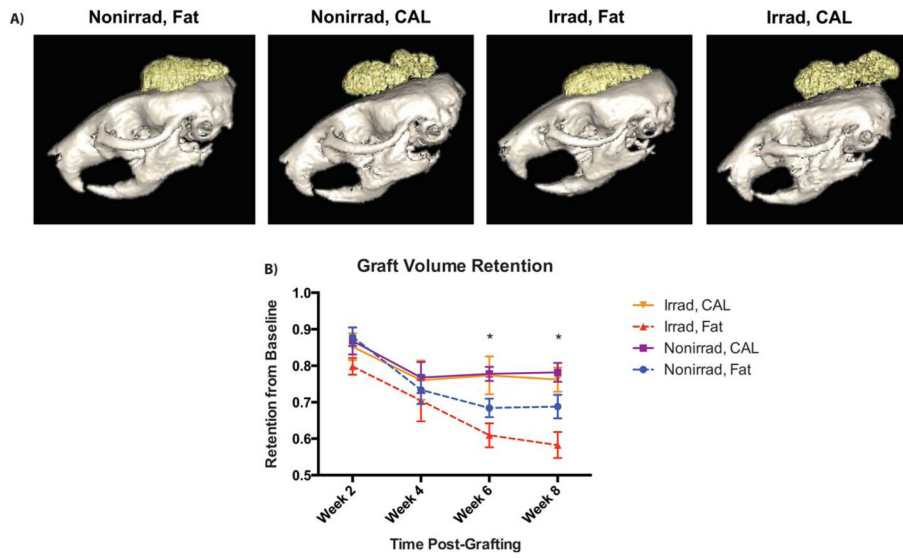


Figure 1. Volume retention of grafts over time. **(A):** Representative reconstructed microcomputed tomography images of fat-only and CAL grafts at week 8 postgrafting. **(B):** Average graft volume retention ($n = 6$ animals per group), expressed as a percentage with respect to baseline volume, measured over 8 weeks. *, $p < .05$. Abbreviation: CAL, cell-assisted lipotransfer.

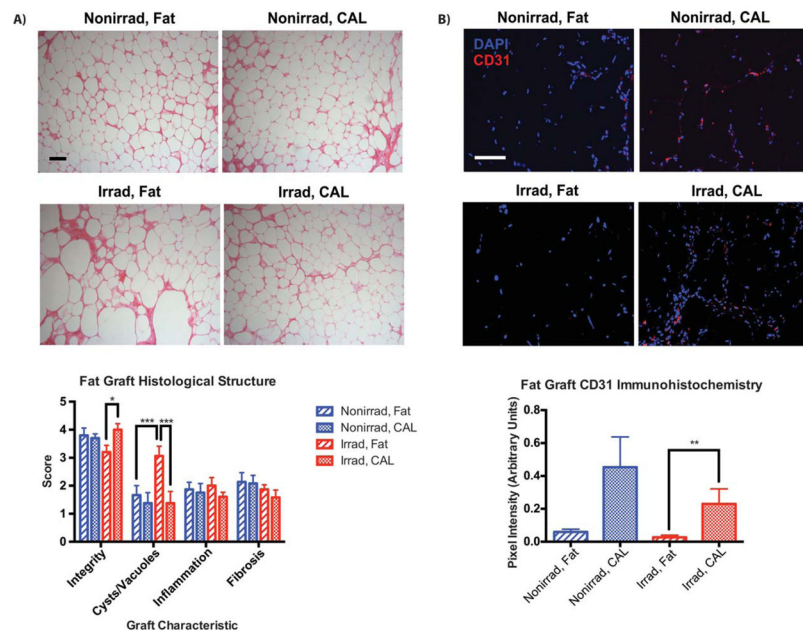


Figure 2. Histological and immunohistochemical assessment of graft structure and vascularity at 8 weeks ($n = 4$ animals per group). **(A)**: Representative H&E staining of sections from fat grafts and CAL grafts with or without prior irradiation of the recipient site, $\times 10$ magnification (top). Scoring of graft architectural characteristics, based on H&E stained sections (bottom). **(B)**: Representative CD31 immunofluorescent staining of fat and CAL grafts, $\times 20$ magnification (top). Quantification of CD31 staining, demonstrating vascularity of grafts (bottom). Scale bar = $100 \mu\text{m}$. *, $p < .05$; **, $p < .01$; ***, $p < .001$. Abbreviations: CAL, cell-assisted lipotransfer; DAPI, 4',6-diamidino-2-phenylindole.

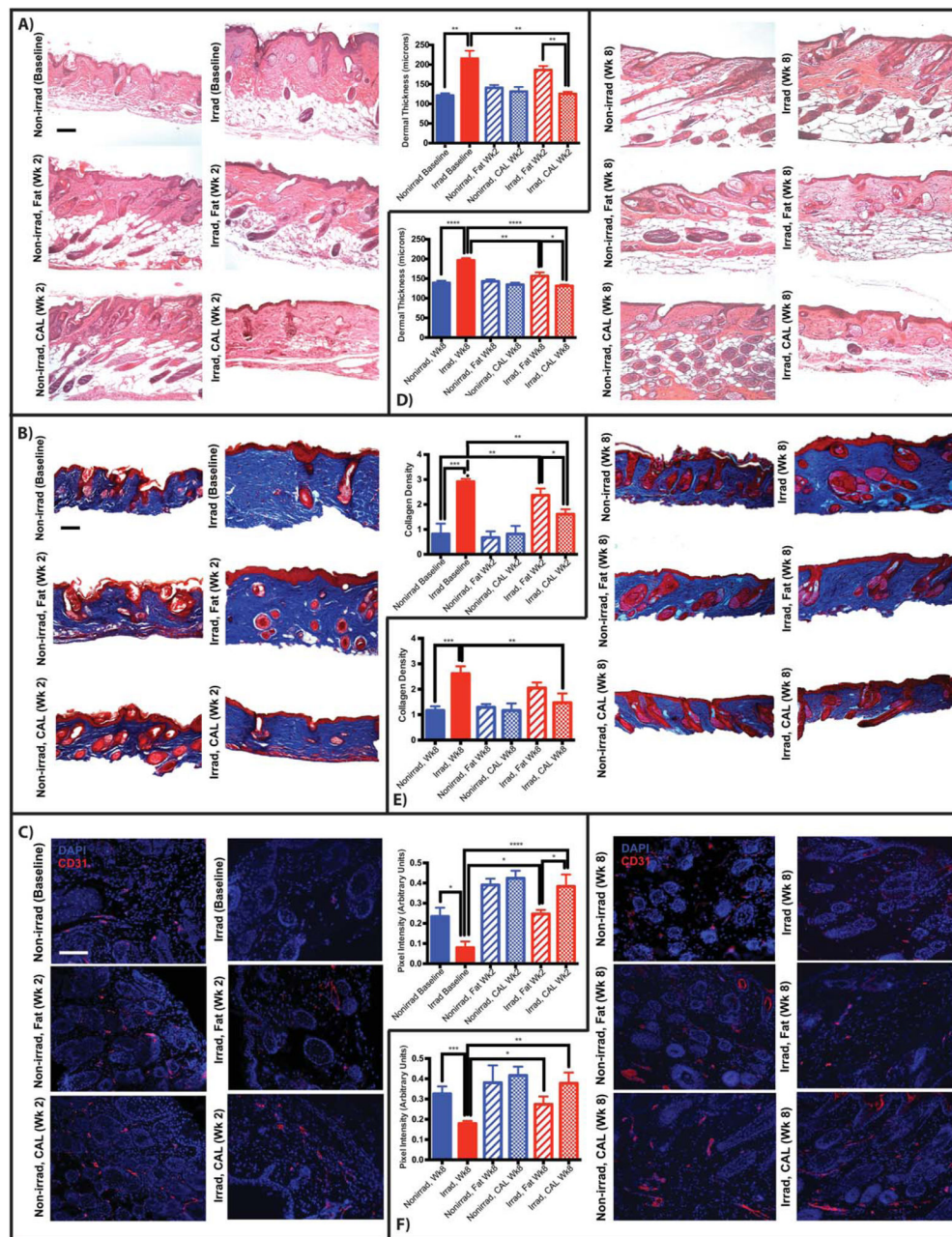
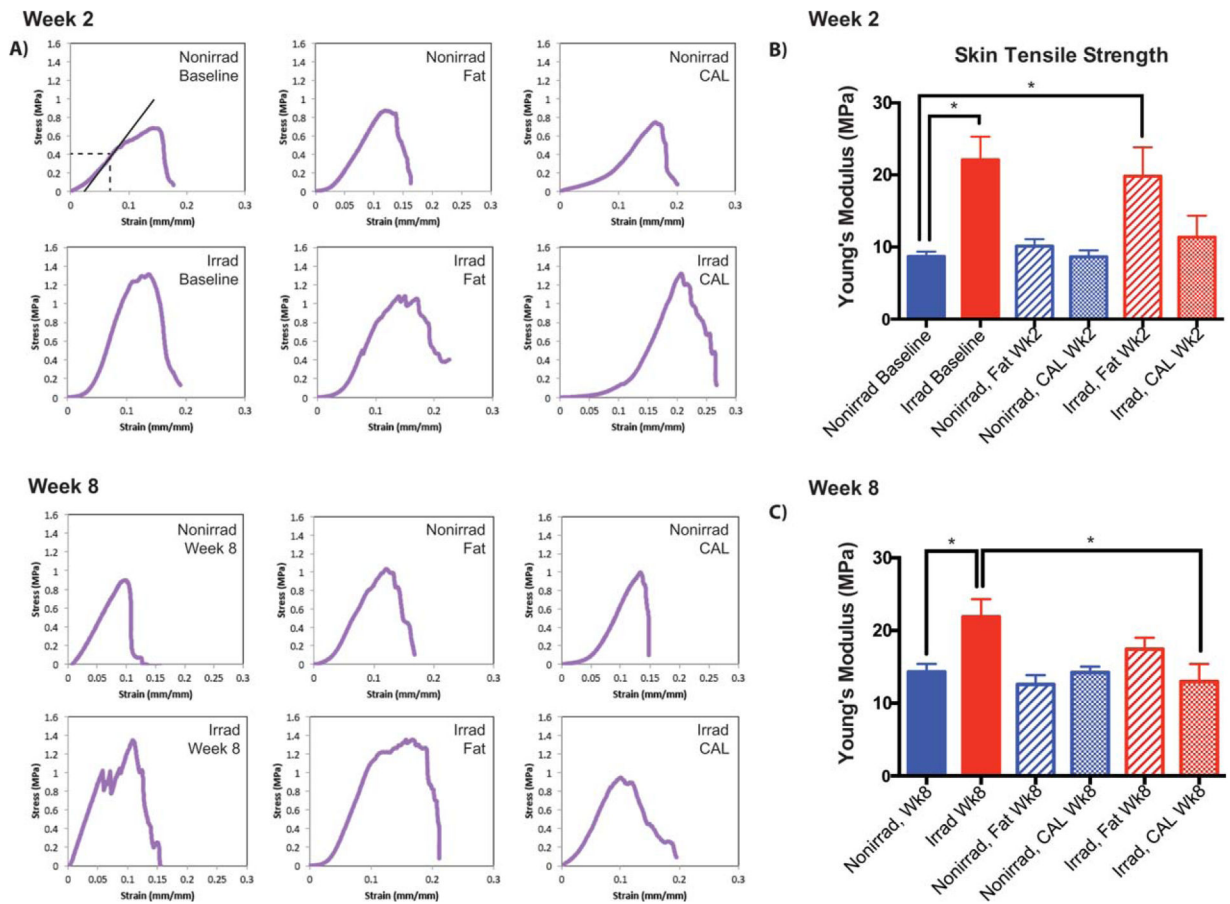


Figure 3. Histological and immunohistochemical assessment of skin ($n = 4$ animals per group). **(A)**: Representative images ($\times 10$ magnification) and dermal thickness derived from two H&E stained sections from the scalp of each animal, each with three measurements at regular intervals, week 2. **(B)**: Representative sections ($\times 10$ magnification) and quantified collagen density of skin stained with Masson's Trichrome, week 2. **(C)**: Representative images ($\times 20$ magnification) and quantification of vascular density of sections stained for CD31, week 2. **(D)**: Dermal thickness, week 8. **(E)**: Collagen content, week 8. **(F)**: Vascular density, week 8. Scale bar = $100 \mu\text{m}$. *, $p < .05$; **, $p < .01$; ***, $p < .001$; ****, $p < .0001$. Abbreviation: CAL, cell-assisted lipotransfer.

**Figure 4.**

Tensile testing of skin overlying grafts ($n = 4$ animals per group). Samples were trimmed to $1.8 \text{ cm} \times 0.5 \text{ cm}$ and positioned into the machine with 8 mm gauge length and crosshead speed moving at a constant rate of 5.1 mm/minute along the sample axis. **(A)**: Representative stress-strain curves of samples, demonstrating measurement of Young's modulus. **(B)**: Young's modulus of irradiated and non-irradiated skin, and either without grafting, 2 weeks after fat grafting, or after CAL grafting. **(C)**: Young's modulus, week 8. *, $p < .05$. Abbreviation: CAL, cell assisted lipotransfer.