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Effect of local, long-term delivery of platelet-derived growth factor (PDGF) on injected fat graft survival in severe combined immunodeficient (SCID) mice

Randall O. Craft [a,b], John Rophael [a,b], Wayne A. Morrison [a,b], Aditya V. Vashi [a,c], Geraldine M. Mitchell [a], Anthony J. Penington [a,b,*]

[a] *Bernard O'Brien Institute of Microsurgery, St. Vincent's Hospital, Fitzroy, Victoria 3065, Australia*
[b] *Department of Surgery, University of Melbourne, Victoria 3010, Australia*
[c] *Department of Chemical and Biomolecular Engineering, University of Melbourne, Victoria 3010, Australia*

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Summary *Background:* Autogenous fat injection is widely used for the correction of acquired and congenital soft tissue defects. However, the high absorption rate results in the need for over-correction of the defect and repeat procedures. We hypothesised that platelet-derived growth factor (PDGF), a potent mitogen and known stimulant for murine preadipocytes, would improve fat graft survival when concentrations were sustained with a gelatine microsphere delivery system.
Methods: Abdominal fat was harvested from an otherwise healthy 43-year-old woman during a breast reconstruction. Prior to subdermal injection into severe combined immunodeficient (SCID) mice, the fat grafts were divided into 1-ml aliquots, mixed with microspheres bound to PDGF, free PDGF, or nothing depending on its experimental group, and weighed. The following experimental groups were thus created (minimum $n = 8$ per group): (1) fat graft control, (2) fat graft with free PDGF, (3) fat graft with blank microspheres, and (4) fat graft with microspheres bound to PDGF. After 12 weeks, the fat xenografts were harvested for analysis of weight maintenance and histological and morphometric evaluation.
Results: The addition of PDGF bound to gelatine microspheres was effective in improving xenograft weight maintenance ($P = 0.018$) and preservation of adipose tissue architecture ($P < 0.0005$) compared to controls at 3 months. The microspheres were completely absorbed at 12 weeks.

* Corresponding author. Address: The Bernard O'Brien Institute of Microsurgery, 45 Fitzroy Street, Fitzroy, Victoria 3065, Australia. Tel.: +61 3 9288 4018; fax: +61 3 9416 0926.
E-mail address: a.penington@unimelb.edu.au (A.J. Penington).

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Conclusions: Sustained, local delivery of PDGF via a gelatine microsphere delivery system resulted in improved weight maintenance of the xenografts with greater preservation of adipose tissue architecture at 3 months compared to controls.

Autologous fat injection is widely used for the correction of acquired and congenital soft tissue defects, most commonly in the face. This technique has been described in the treatment of facial lipodystrophy, augmentation of nasolabial furrows, depressed scars, post-traumatic defects, and enlargement of the lips.[1] This method is also used in the treatment of atrophic cutaneous tissue on the dorsum of the hand, as well as correction of contour defects resulting from liposuction. The abundance of adipose tissue in most individuals, combined with the low morbidity and versatility of the procedure, has made autologous fat grafting an attractive option for soft tissue augmentation for over a century.[2]

The major drawback of fat transplantation is its variable longevity, with up to 70% of the injected volume ultimately being absorbed.[3–6] The high absorption rate results in the need for over-correction of the defect and repeat procedures, with each additional procedure further increasing the risk of graft failure and absorption by damaging the blood supply to the recipient area.[7] The inconsistency in long-term results led to a focus on the differences in the diverse methods of harvesting, treatment, and placement of the fat.[8] Subsequently, a multitude of methodological variations have been described including atraumatic harvesting of fat with low-pressure suction,[8,9] avoidance of graft contact with room air,[8] fat concentration procedures,[10] and washing of the graft to remove inflammatory mediators.[11] However, a recent animal model has demonstrated that none of the common methods of harvesting or preparing fat result in consistently increased graft viability,[12] leaving unpredictable long-term success a major obstacle to autologous fat grafting.[13]

Recent years have seen a host of studies describing graft manipulations intended to affect preadipocytes, mature adipocytes, or the recipient bed in an attempt to use 'bioactivation' of harvested fat to improve long-term outcomes.[7,14–16] Treatments have included addition of basic fibroblast growth factor (bFGF) attached to dextran beads,[14] insulin, insulin-like growth factor, and bFGF delivered through a poly (lactic-co-glycolic-acid)-polyethylene glycol (PLGA/PEG) microsphere delivery system,[16] and interleukin-8,[7] all with varying degrees of success.

Platelet-derived growth factor (PDGF) is a potent mitogen of mesenchymally-derived progenitor cells,[17] and has been shown in vitro to stimulate growth of murine preadipocytes.[18] Independent of its stimulatory role, PDGF has also been shown to have an anti-apoptotic effect on this cell population in serum-free culture conditions.[19]

In the present study, we evaluated the effect of local, long-term delivery of PDGF via a gelatine microsphere delivery system on fat graft weight maintenance and histological composition to determine if these effects translate to an in vivo model. We demonstrate that the sustained delivery of PDGF successfully improves the weight maintenance and cellular architecture of human fat subdermally placed in severe combined immunodeficient (SCID) mice at 12 weeks.

Materials and methods

Animals

All experiments were performed with the prior approval of the St. Vincent's Hospital, Melbourne, Animal Ethics Committee, under the National Health and Medical Research Council Guidelines (Australia). Severe combined immune deficient (SCID) mice weighing between 20 and 25 g were used for this experiment. The SCID mouse model allows for the study of transplanted human fat without the immunological sequelae of rejection.[20]

Fat graft and experimental design

Fat was harvested by excision from the abdomen of an otherwise healthy 43-year-old woman during a breast reconstruction performed under general anaesthesia. The tissue was washed twice with sterile phosphate-buffered saline, sectioned into small pieces with scissors, and then passed repeatedly through a 3-ml syringe attached to a 14-gauge needle until it reached a gelatinous consistency to simulate the mechanical disruption of cannular harvesting, as previously described in a related model.[14]

The pericranial region of the mouse was chosen as the recipient site because of the absence of native fat in this area. Graft implantation was done by percutaneous injection (14-gauge needle) under general anaesthesia (chloral hydrate administered intraperitoneally at 0.4 mg/g body weight) (Figure 1). Prior to injection, the fat grafts were divided into 1-ml aliquots, mixed with microspheres, free PDGF, or nothing depending on the experimental group, and weighed. The following experimental groups were thus created (minimum $n = 8$ per group): (1) fat graft with microspheres bound to PDGF, (2) fat graft alone, (3) fat graft with free PDGF, and (4) fat graft with blank microspheres. All grafts were harvested 12 weeks after graft insertion and evaluated for weight maintenance, histological changes, and histomorphometry.

PGDF-BB

Recombinant rat platelet-derived growth factor-bb (rr PDGF-BB) was purchased from R&D Systems (Minneapolis, MN, USA). The growth factors were reconstituted to a final concentration of 50 μg/ml according to the manufacturer's specifications.