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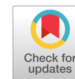
Should platelet-rich plasma be activated in fat grafts? An animal study



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Summary *Background:* The adjunction of platelet-rich plasma with graft fat has been the subject of a few clinical trials which have demonstrated its value in adipocyte survival. The aim of this study was to assess the different efficacies between activated and non-activated PRP on adipose cells *in vitro* and for adipose tissue graft survival *in vivo*.
Methods: The *in vitro* study assessed the effects of PRP on both the proliferation and adipocyte differentiation of adipose cells.

For the *in vivo* study, 8 nude rats received 3 human fat injections as follows: 0.8 mL of fat + 0.2 mL of normal saline; 0.8 mL of fat + 0.2 mL of non-activated PRP; and 0.8 mL of fat + 0.2 mL of PRP activated with calcium chloride ($CaCl_2$).

The quantitative assessment of adipocyte survival was implemented after 3 months using histomorphometric analysis. Histological and immunohistochemical analysis were also performed to evaluate angiogenesis, inflammation and quality of adipocytes in the grafted tissue.
Results: We showed that activated PRP stimulated, *in vitro*, proliferation and differentiation of adipose cells.

In vivo experiments indicated that $CaCl_2$-activated PRP was more efficient than non-activated to prolong the survival of fat grafts in nude rats. The mean percentage areas occupied by viable adipocytes in the PRP-free group, non-activated PRP group and activated PRP group were 13%, 14% and 24% ($p = 0.05\%$), respectively. Histological and immunohistochemical analysis revealed protective effect of activated PRP on inflammation and adipocyte death.

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Conclusion: This study showed that activation by $CaCl_2$ improves the beneficial effects of PRP for fat graft maintenance.
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Introduction

Autologous fat transfer is an increasingly widely used technique in plastic surgery for the reconstruction of soft tissue defects that may be secondary to tumor excision, congenital malformations or post-traumatic sequelae. This simple and minimally invasive technique is nonetheless associated with a major disadvantage related to the uncertain nature of fat transplant survival. The mean survival rate of injected fat, at 3 months, is estimated to be between 40% and 60%,[1,2] depending on the study, resulting in the need to repeat procedures.

Several strategies have been studied with a view to increasing adipocyte survival, such as the adjunction of angiogenic growth factors or stromal vascular fraction, or use of chemical cell-stimulating factors such as insulin or erythropoietin.[3–8]

The adjunction of platelet-rich plasma (PRP) has been the subject of a few clinical trials which have demonstrated its value in adipocyte survival.[9,10] The evidence for combining PRP with fat grafts nonetheless remained limited.

Platelet-rich plasma consists in a small volume of plasma with a high platelet concentration (3- to 5-fold the baseline platelet count). The alpha granules of platelets contain numerous growth factors and adhesion molecules.

The regenerative and cicatrizing properties of PRP are related to its ability to secrete those proteins in the course of the platelet activation process.[11,12]

Activation consists in the degranulation process in which alpha granules fuse with the platelet membrane thus releasing secretory proteins.

In clinical practice, PRP may be used in activated or non-activated form.

Various methods of PRP activation have been developed. Eppley et al, Kim et al and Marx et al have reported an activation process which consists in mixing 6 mL of PRP with 1 mL of calcium chloride ($CaCl_2$) plus thrombin. This activation enables release of growth factors over an hour.[11,13,14] Another activation technique consists in mixing PRP with $CaCl_2$ only, without thrombin, enabling formation of a fibrin matrix surrounding the growth factors which will thus be released in a very gradual manner over a timeframe of 7 days.[15] The latter activation method was assessed in the context of our study.

To date, no study has compared activated PRP and non-activated PRP in fat grafts.

We hypothesized that calcium chloride-activated platelets are more effective in fat grafting than non-activated platelets. In order to investigate this hypothesis, we first conducted an *in vitro* study to assess the effects of PRP in absence of or following treatment with $CaCl_2$ on both the proliferation of adipose-derived stem cells and their differentiation into mature adipocytes. We then compared the effects of activated and non-activated platelets for fat graft retention in nude rats. Histological and immunohistochemical analysis were used to characterize exogenous adipose tissue following different engraftment conditions.

Materials and methods

In vitro study

Isolation and culture of human adipose stem cells

The human adipose stem cells were isolated from different young donors with written informed consent of the parents, as surgical scraps from surgical specimens obtained from various surgeries, as approved by the Regional Ethical Committee ("Comité de Protection des Personnes Ile de France IX"). The human adipose stem cells were isolated as previously described.[16] Briefly, 200 mg/mL adipose tissue was dissociated for 5–10 min in Dulbecco's modified Eagle's medium (DMEM) containing antibiotics (100 U/mL of penicillin and 100 μg/mL of streptomycin), 2 mg/mL collagenase, and 20 mg/mL bovine serum-albumin. The crude stromal vascular fraction (SVF) was separated from the adipocyte fraction by low speed centrifugation (200 *g*, 10 min). The adipocyte fraction was discarded and cells from the pelleted SVF were seeded onto uncoated tissue culture plates (Greiner) at 1000–3500 cells/cm^2 in low glucose DMEM (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (D. Dutscher) and antibiotics as described before.

The human adipose stem cells were cultured in DMEM, 1 g/L glucose containing 10% FBS (D. Dutscher), 100 U/mL penicillin, 100 μg/mL streptomycin, and 10 mM HEPES (Invitrogen) in a 5% CO_2 atmosphere at 37 °C. As described by Rodriguez et al, human adipose stem cells had the following phenotype: $CD44^+$, $CD49b^+$, $CD105^+$, $CD90^+$, $CD13^+$, Stro-1$^-$, $CD34^-$, $CD15^-$, $CD117^-$, Flk-1$^-$, Gly-A$^-$, $CD133^-$, HLA-DR$^-$ and HLA-I^{low} . These cells are multipotent and are able to differentiate into adipocytes, osteoblasts, chondrocytes, endothelial cells and muscle cells.[16]

For the present study, we used these cells as model of human pre-adipocytes as they have been reported to be able to differentiate into functional adipocytes.[17]

Proliferation assay

For the proliferation assay, 5000 human adipose-derived stem cells/well were seeded in a 24-well plate and grown under 9 different culture conditions (Supplementary Figure S1): FBS-free DMEM (negative control); DMEM + FBS (positive control); DMEM + $CaCl_2$ (positive control 10%); DMEM + 5% of PRP; DMEM + 10% of PRP; DMEM + 20% of PRP; DMEM + 5% of PRP + $CaCl_2$; DMEM + 10% of PRP + $CaCl_2$; DMEM + 20% of PRP + $CaCl_2$.

All conditions were assessed in triplicate. Thus, for each experiment, 27 wells were seeded in two different plates as shown in Supplementary Figure S1 and all experiments were repeated three times.

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