Journal of Plastic, Reconstructive & Aesthetic Surgery (2016) 69, 952—958

JPRAS
An International Journal of Surgical Reconstruction
www.JPRASurg.com

# Autologous plasma and its supporting role in fat graft survival: A relevant vector to counteract resorption in lipofilling

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Received 6 July 2015; accepted 18 March 2016

**KEYWORDS**
Plasmatic imbibition;
Lipofilling;
Fat graft;
Plasma;
Resorption

**Summary** Fat grafting has become a widespread technique for different reconstructive and esthetic purposes. However, the disadvantage of fat grafting is the unpredictable resorption rate that often necessitates repetitive procedures, which in turn may have an impact on the morbidity. During the immediate, post-graft, ischemic period, cells survive due to the process of plasmatic imbibition. This biological phenomenon precedes the ingrowth of neo-capillaries that eventually nourish the graft and help establish a long-term homeostatic equilibrium. Both partners, the graft and the recipient bed, contribute to the revascularization process. Hypothetically, enrichment of the recipient site with autologous plasma could have a beneficial role to enhance fat graft survival. We investigated whether plasma supported the viability of the lipoaspirate (LA) material. Plasma was isolated from blood samples collected from eight patients during the elective lipofilling procedures. An in vitro study assessed the viability of LA cells using plasma as a culture medium compared to the traditional culture media. In vitro analysis confirmed sustained viability of LA cells compared to the standard media and control media during 7 consecutive days. The behavior of the fat grafts in plasma showed similarities with those incubated in the traditional culture media. In future, these findings could be translated to a clinical setting. Plasma is the only autologous substrate available in large quantities in the human body. The addition of the supporting agents, such as plasma, could contribute to a better graft survival with more stable clinical outcomes in the long term. The rationale behind the

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http://dx.doi.org/10.1016/j.bjps.2016.03.014

technique is based on the phenomenon of plasmatic imbibition and the reasoning that the extracellular matrix plays a pivotal role in cellular survival.


## Introduction

The concern of fat grafting is the variable rate of resorption. Fat grafting succeeds at a microscale level by providing the appropriate cell type in a theoretically suitable microenvironment, but it often fails at a macroscale level, because a part of the transplanted cells is located >150 μm away from the blood vessels, which is the diffusion limit for oxygen and nutrients (Figure 1).[1] The biological phenomenon of plasmatic imbibition supports grafted cells to survive the immediate post-graft ischemic period.[1,2] The graft "drinks" plasma and absorbs nutrients to protect itself from desiccation. The post-graft period is undetermined. It varies depending on the tissue quality at the recipient site as well as the quality of the graft. For a skin graft, this may be up to 24 or even 48 h.[1,2] Scientific data regarding the immediate post-graft period of transplanted fat are inadequate. However, we can assume that plasmatic nutrition plays a crucial role and dictates the clinical outcome. It is well known that the adipose organ is a highly vascularized organ, and a rapid and efficient revascularization is crucial for fat graft survival.[3,4] The intention of lipofilling is to place a graft in a steady-state, homeostatic equilibrium. Homeostasis means that the graft has a well-balanced physiological interaction with its microenvironment. Rather than the addition of stimulatory agents, "supporting" agents would be superior to achieve this equilibrium without additional stimulation at a cellular level.[5] We were able to generate three-dimensional (3D) adipose constructs in rodents and observe the process of angiogenesis with the close relationship between angiogenesis and developing adipocytes.[6] Analysis of these data has contributed substantially to the understanding of fat graft behavior in vivo. The matrix is the main vector in processes of cellular migration, proliferation, and differentiation. It coordinates cell—cell and cell—matrix interactions in a geometrical 3D framework.[7—11] The lipofilling procedure is basically a "reversed reconstructive approach": a biological disharmony exists between the graft and the recipient site, as both need to contribute to the revascularization process crucial for achieving homeostasis.[1,2] Liposuction disrupts the extracellular matrix (ECM), and the subsequent processing of the lipoaspirate (LA) material jeopardizes cell viability due to a considerable degree of trauma.[12,13] The injection of this LA material places the graft in a new environment that lacks substantial nutritional support, whilst plasmatic imbibition will also have its spatial (graft volume) and temporal (post-graft period) limits.[1,2,14—16] The main determinant for cell survival is an appropriate, supportive microenvironment.[3—9,14—16] Following the "replace-like-with-like" principle and the prospectus of a future based on autologous approaches, we are considering plasma as a potential nutritional vehicle for fat grafts. This paper reviews the immediate post-graft period in fat grafting and hypothesizes the use of plasma as a medium to support fat graft viability and counteract resorption.

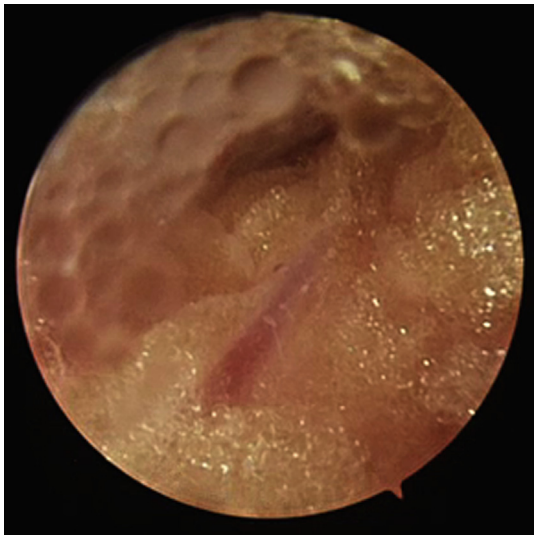

**Figure 1** Endoscopic view after a lipofilling session: fat grafts are observed in proximity with a capillary. Those grafts survive the immediate post-graft period through plasmatic imbibition and before neo-angiogenesis, they are released from their ischemic condition.

## Materials and methods

### In vitro study

#### Tissue harvest and preparation

Preoperatively, 20 cc of heparinized blood (0.1 cc per 100 ml of blood) was obtained from the patient and centrifuged for 7 min at 3000 rpm (1200 G; Figure 2, left). The supernatant plasma was aspirated and stored in a 10-cc Luer lock syringe (Figure 2, right). Lipoaspirate (LA) material was obtained with informed patient consent through the Plastic and Reconstructive Surgery Unit at the University Hospital Gent (Ethics Committee number EC 2010/496). The fat was harvested using the Coleman technique from the thigh region. The donor sites were infiltrated with Klein solution using a blunt infiltrator. A 3-mm harvesting cannula was used, connected to a 10-cc Luer lock syringe, which was spun at 3000 rpm for 3 min (1200 G; Byron Medical Inc.,

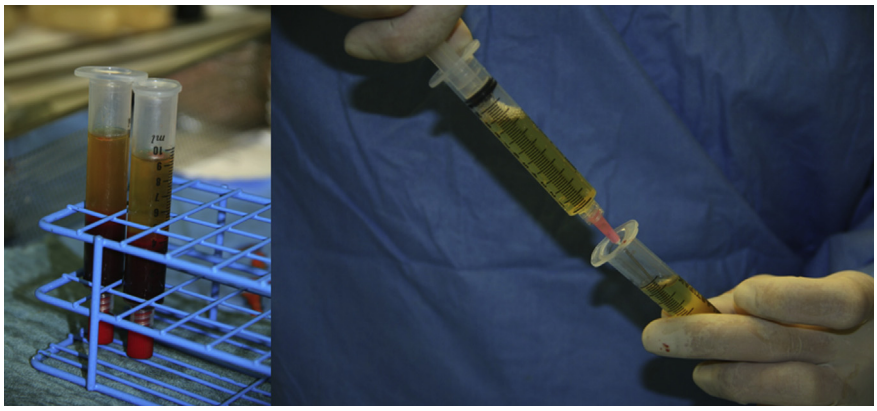

**Figure 2** A heparinized blood sample was centrifuged for 7 min at 3000 rpm (1200 G) to separate the plasma fraction (left). The plasma fraction is aspirated and stored in Luer lock syringes of 10 cc ready for injection (right).

Tucson, AZ). The oil layer was decanted and the aqueous layer drained out of the syringe. The bottom layer was used for the in vitro study. Both plasma and LA material were transported in aseptic conditions without cooling.

### Cell cultures

The LA material (1 ml per well) was placed in 12-well culture plates (Nunc). The wells were filled with culture medium (1 ml) to cover the LA material. Four culture media were compared: autologous plasma (group 1), fetal calf serum (FCS, Gibco Invitrogen; group 2), Dulbecco's modified Eagle Medium (DMEM, Gibco Invitrogen) enriched with 10% FCS (group 3), and DMEM alone (group 4). The control media were glucose 5% (Hartman; group 5) and NaCl 0.9% (Baxter; group 6). The plates were incubated at 37 °C on a gyratory shaker (Gerhardt, Laboshake) for 7 days. Culture media were not changed and no additional growth factors or antibiotics were added. Cell viability and leptin expression were assessed daily. The experiment was performed with eight patients in triplicate.

### Cell viability (Alamar Blue assay)

Cell viability and proliferation were quantified using the Alamar Blue assay (Invitrogen). To perform the Alamar Blue assay, LA was cultured in triplicate for each time point (seven time points). At each day, 300 μl from each well was retrieved for the leptin analysis. Alamar Blue reagent (170 μl) was added to each well containing the residual 1.7 ml LA (2.0 ml−300 μl). The well plate was incubated at 37 °C on a gyratory shaker for 2 h. Approximately 100 μl was retrieved from each well and transferred to a 96-well plate. The LA/Alamar Blue mixture was discarded at each time point. The fluorescence intensity was performed on the Wallac 1420 Viktor 3™ plate reader (Perkin Elmer, Inc.) at 525 nm (excitation)/615 nm (emission). The procedure was performed daily from day 0 to day 7. Results are expressed as % viability relative to day 0. The mean and standard error of mean (SEM) of seven patients are calculated in triplicate.

### Leptin concentration (enzyme-linked immunosorbent assay)

Approximately 300 μl was filtered from each well (0.22 μm, Millipore) and the retrieved media were frozen at −18 °C for analysis. Enzyme-linked immunosorbent assay (ELISA) analysis was performed with the Human Leptin Duoset kit (R&D systems) according to the manufacturer's protocol.

### Statistics

Results are presented in the form of mean plus twice the standard error. We used a mixed model test with EXPERIMENT as the random factor; hence, the variation between the experiments is considered. We applied the Bonferoni correction for multiple testing. This brings the significance level from 0.05 to 0.00333.

## Results

### In vitro study

#### Alamar Blue staining (Figure 3)

The standard culture media DMEM, DMEM−FCS 10%, and FCS sustained the viability of the inoculated cells with a peak at day 5. All three culture media supported cell viability during the 7 consecutive days as expected from those culture media. LA material cultured in 0.9% NaCl initially showed a rapid decrease in cell viability at day 1 followed by a progressive decrease and absence (<10%) in viability at day 7. The wells containing 5% glucose supported the cell viability until day 2, but this viability reduced considerably at day 3. Comparable to the standard culture media, plasma showed a steady support of cell viability during the 7-day period. The initial viability measurement at day 0 remained constant during the 7 days and increased slightly at day 3.

#### Leptin expression (Figure 4)

Leptin is strongly expressed in white adipose tissue and absent or expressed at extremely low levels in other tissues.[17] Leptin expression increases in parallel to the adipose stores as it is a readout signal of adipocyte triglyceride stores.[17] The initial leptin expression at day 0 was practically the same for all groups. At day 1, the plasma group showed a peak in leptin expression compared to the other groups. A slight increase in leptin expression was only observed in the DMEM group. This DMEM group showed the

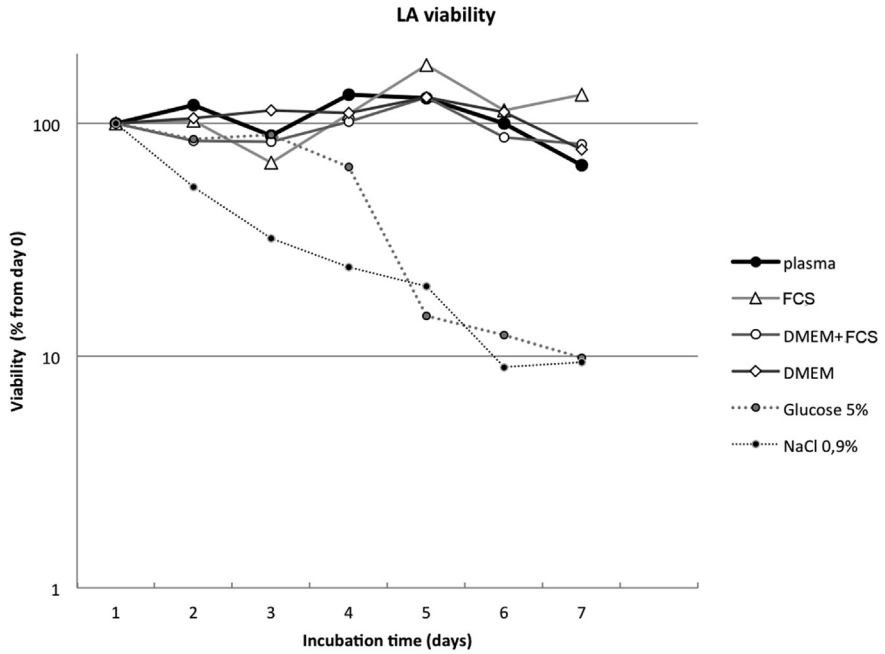


**Figure 3** Viability of cells within the lipoaspirate material was measured using the Alamar Blue staining. Compared to both control groups (glucose 5% and NaCl 0.9%), plasma showed a sustained viability of the lipoaspirate cells. At day 5, viability in plasma was identical to DMEM + FCS and DMEM. Fetal calf serum showed the highest viability at day 5 and finally at day 7. Cell viability in plasma followed almost exactly the same pattern as DMEM with and without FCS.

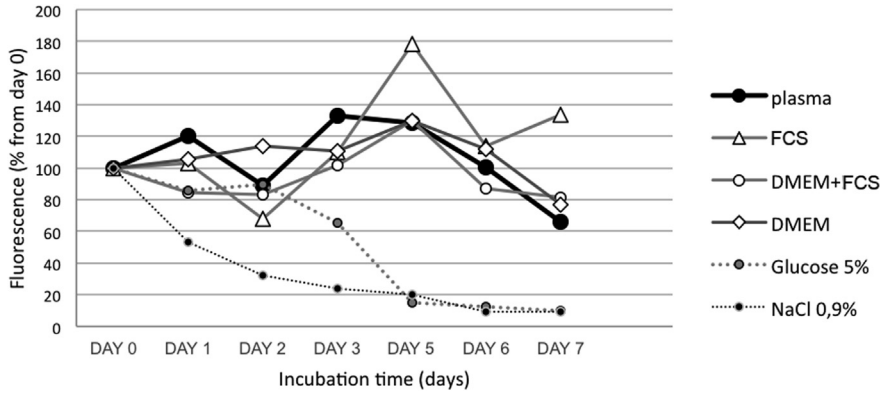


**Figure 4** Leptin expression of the lipoaspirate cells cultured in plasma peaked at day 6. This peak of leptin expression at that specific day was similar to the FKS group and the DMEM + FKS group.

same peak only at day 2, whilst the leptin expression in the plasma and FCS groups showed the same drop at that time period. However, at day 3, the plasma group showed a significant increase again in expression that was higher compared to the FCS and DMEM groups, thereby suggesting a more substantial nutritional support of this medium in adipocyte development. This cannot be explained by a cumulative effect because the half-life of leptin is less than 1 day (24.9 $\pm$ 4.4 min). This peak in leptin expression at day 3 is probably because precursors or preadipocytes undergo further differentiation into mature adipocytes with triglyceride storage, a process supported by the presence of a supportive medium. This peak has not been observed in the control groups and was statistically significant ($p < 0.001$). At day 5, the FCS group showed the highest leptin expression compared to all groups. Culture media with plasma, DMEM + FCS 10%, and DMEM alone showed the same expression values. Expression was completely absent in the control groups (glucose 5%, NaCl 0.9%) at day 5. At day 6, leptin expression was again at the same level of day 0 in the plasma group and decreased in the following time periods. The same observation was made for the other groups except for the FCS group that showed an increase at day 7.

## Discussion

The unpredictable resorption rate of grafted fat often results in patient dissatisfaction and necessitates repeated

procedures.[15,18,19] The adipose organ is a dynamic, connective tissue composed of a heterogeneous cell population and a dense microvascular system entwined within a "biological glue": the ECM.[10,11] This ECM embeds cells in a nutritional and structural micro-environment that dictates cell behavior and responds to the functional demands of the cells.[7,10,11] Essentially, fat grafting is a rather reversed approach to tissue regeneration/augmentation. The resorption phenomenon, inherent to this technique, is initially and mainly coordinated by the post-graft ischemic period during which transplanted cells need to survive through a process of diffusion.[15,16,18] The final outcome of fat transplantation is subsequently determined by an efficient and rapid revascularization of the graft that occurs already at day 1.[12,14–16,18,20] Langer observed vascular sprouts at the border of the graft at that time period in rodents with a functional vessel density value comparable with that of normal fat tissue at day 12.[12] In an effort to counteract the resorption rate in fat grafting, surgeons have been adding additives to the graft or have been trying to manipulate the recipient site or environment.[20–25] Fat grafting is an autologous-based surgical technique and it is our belief that this autologous-based character should be respected in order to relief the biological disharmony between the graft and the recipient site, to avoid foreign body reactions or inflammation and to achieve tissue homeostasis in the long term. In our opinion, it might be controversial to add growth factors to the fat graft, which contains stem cell precursors. From a practical point of view, the only tissue in the human body, which is easily available and accessible, is the peripheral blood pool. Plasma is the main component of this largely available tissue and involved in the biological phenomenon of plasmatic or serum imbibition.[1,2] The idea to use autologous plasma as a supportive vehicle originated not only from the knowledge of this biological phenomenon but also from reports of in vitro studies trying to replace the standard expansion culture media supplements, such as fetal bovine (FBS) or FCS with plasma.[28–38] Our results clearly show that plasma supports the survival of the fat graft in vitro the first consecutive 6 days. This transient period is extremely important to protect the fat graft from dehydration and nutritional deficit before revascularization occurs that links sprouting capillaries between the graft and the recipient site. It has also been shown that, in skin grafts, a preparation phenomenon exists where the graft stimulates the angiogenic induction of the recipient site.[2] In this regard, an additional advantage of fat grafts is their highly vasculogenic potential and the preparation phenomenon could be more pronounced in fat grafts due to this vasculogenic activity. Adipose tissue is a potent source of secreted cytokines such as VEGF (vascular endothelial growth factor) and leptin.[17,26,27] The data of the leptin expression study show the expression of two peaks of expression. This is not due to a cumulative effect because of the short half-life of leptin. The second peak in expression occurs at day 3 in the plasma group which is a day earlier than the other groups. This is probably because young adipocytes or precursors store triglycerides, thus confirming the survival of those cells in the plasma medium. Leptin expression remained remarkedly steady during the 7 consecutive days compared to the other groups. It makes plasma a very potential substrate for cells to survive with in vivo as well as in vitro applications. From a hypothetical clinical point of view, the recipient site could be infiltrated with plasma before the grafting procedure is performed (Figure 5). Plasma added to the recipient site could play a supportive role in fat graft survival, essentially in the immediate postoperative period. Currently, we are investigating this plasma-based working algorithm in our fat grafting procedures, and further studies are scheduled to analyze the potential beneficial influence on clinical outcomes. The ratio of injected plasma versus LA is 1/3 and injection of the plasma does not seem to have a negative impact on the interstitial pressure or the amount of injected volume of fat. This in vitro study is a strong and reliable "proof-of-principle" concept that opens new perspectives in our search to enhance the survival rates of the fat grafting procedures, to decrease the total number of procedures, and to develop new scaffolds that are devoid of growth factors or stimulatory agents. Rigid or semirigid biological scaffolds are not an option because embryologically, adipogenesis and angiogenesis are strongly related to each other. A scaffold in adipose tissue engineering strategies should have liquid or semiliquid characteristics that support cellular migration as well as cellular anchoring and allow a concomitant development of a vascular plexus. The next step in our search for the ideal matrix would be the alteration of the consistency of the plasma component into a more biodegradable scaffold that anchors the fat grafts and allows ingrowth of neocapillaries. This preferentially nonrigid, plasma-based scaffold would mimic the more structural microenvironment of the adipose tissue. This study shows the potential and practical ease of plasma-based fat grafting: plasma can

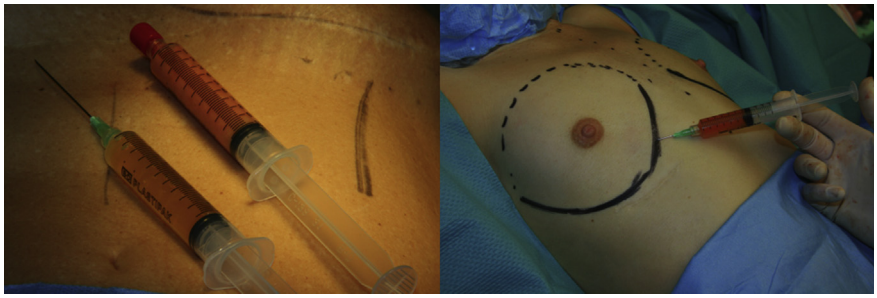

**Figure 5** Plasma and lipoaspirate material are collected in 10-cc syringes and ready for injection (left). The recipient site is infiltrated first with plasma in the subdermal layers in a fan-like pattern (right).

easily be obtained and processed in the operating room without the need of expensive processing devices. It should be clear that this potential working algorithm differs from the platelet-enriched plasma (PRP) procedures that necessitate a time-consuming and cost-related processing. Moreover, scientific data are not consistent regarding the efficiency of PRP-enriched lipofillings.[25] PRP gel can only be obtained in smaller quantities insufficient to support large-volume lipofilling sessions.

## Conclusion

Our in vitro research confirms the supportive role of autologous plasma in the survival of the fat grafts. Viability has been observed during 6 consecutive days, and when translated to a clinical setting infiltration of the recipient site could have a beneficial effect on the survival rate of grafted fat. Currently, we are investigating this potential beneficial impact in clinical applications and the ongoing research will focus on the development of new scaffolds based on plasma as a largely available and storable autologous substrate.

## Conflict of interest statement

None.

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