

# Autologous Platelet-Rich Plasma as an Adipocyte *In Vivo* Delivery System: Case Report

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**Abstract** Tissue engineering has emerged as a promising alternative to current clinical treatments for restoration of soft tissue defects. A key element in the process of tissue engineering is an ideal implant that provides structural support and a favorable environment for growing cells. The authors hypothesized that autologous platelet-rich plasma (APRP) could be used as an *in vivo* adipocyte delivery system to favor cell survival and to stimulate early recruitment of microcapillaries to the site of implantation. Autologous fat was included in APRP and injected as a gel into a subcutaneous pocket created to correct a painful, adherent scar at the shoulder level in a 75-year-old woman. The surgical outcome was evaluated by histologic and immunohistochemical analysis as well as by ecography before and after surgery. The results were satisfactory, showing fat survival 1 year after surgery. The characteristics of this new material should stimulate research into future clinical applications for such cell constructs in plastic and reconstructive surgery.

**Keywords** Adipose tissue engineering ·  
Autologous platelet-rich plasma · Filler ·  
Regenerative medicine

Autologous fat transplantation is a procedure increasingly applied for augmentation of soft tissue defects [2, 6, 10].

Currently, plastic surgeons across the country report uniform autologous fat grafting techniques with acceptable patient satisfaction [6]. Fat grafts collected by liposuction can be reinjected subcutaneously for correction of depressed or irregular areas [2, 6, 10]. The live fat tissue is revascularized at the transplantation site within 48 h. During this time, it is fed by diffused materials from plasma. In contrast, nonviable tissue is removed by macrophages, leaving behind fibrotic and cystic changes.

The main obstacle preventing permanent augmentation is partial absorption of the transplanted tissue, which often necessitates multiple operations [2, 6, 10]. A greater understanding of how to maintain viable fat has led to modifications in technique believed to improve clinical results. These modifications are intended to preserve the delicate structure of adipocytes, providing a robust blood supply on which fat cells are extremely dependent or creating the optimal *in vivo* microenvironment for cell growth and differentiation [1, 4, 5, 9].

We decided to use autologous platelet-rich plasma (APRP) to obtain an easy-to-handle biologic gel that could be mixed with adipocytes. The clinical use of APRP for a wide variety of applications has been reported, most prevalently in the problematic wound, maxillofacial, and spine literature, but, to our knowledge, it is never been used as an adipocyte delivery system for *in vivo* tissue-engineering applications [3].

Numerous proteins are contained in the  $\alpha$ -granules of platelets: platelet-derived growth factor, transforming growth factor, platelet factor, interleukin, platelet-derived angiogenesis factor, vascular endothelial growth factor, platelet-derived endothelial growth factor, insulin-like growth factor, fibronectin, and the like [3]. These proteins could stimulate revascularization of the implanted adipocyte-rich gel and constitute a three-dimensional matrix that

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allows for the arrangement of adipocytes into the correct spatial organization. In this study, we present a clinical case in which adipocytes, harvested with traditional liposuction, were mixed with APRP and injected into a subcutaneous pocket to correct a painful adherent scar.

## Case Report

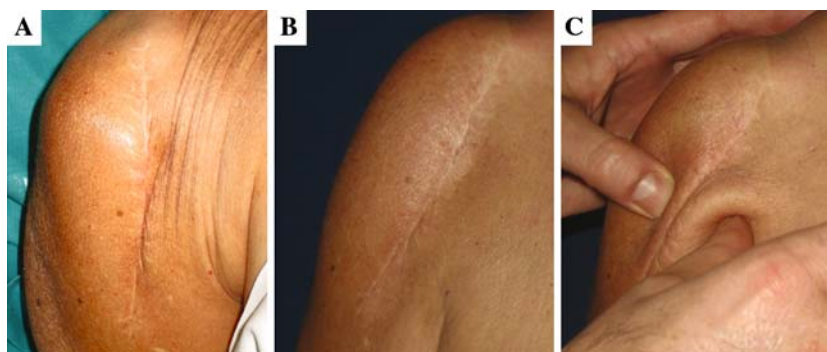
Patient anonymity was respected and informed consent obtained before the surgical procedure and digital image production. The protocol of the study was approved by the research ethics board of our institution.

A 75-year-old woman presented with an adherent and painful scar on the right shoulder (Fig. 1A). Her medical history showed shoulder replacement surgery 2 years previously. She reported the beginning of painful symptoms 6 months after surgery. Pain was localized to the superficial layers and did not involve the shoulder joint. Physical examination showed an 8-cm-long scar adherent to the underlying plane. Magnetic resonance imaging excluded prosthesis defects. Ultrasonography confirmed the direct contact of the scar with underlying fascia, showing an incisure of the skin created by scar tissue (Fig. 2A). We decided to treat the defect with structured fat grafting following Coleman's observations, which noted that fat grafted under depressed scars not only relieved the depression but also seemed to soften or even completely eliminate the scar, making it appear as normal skin [2]. We thought to mix harvested adipose tissue with APRP to improve long-term results. Surgery was performed with the patient under sedation.

## Adipose Tissue Harvesting

We infiltrated the abdomen with 250 ml of wetting solution (50 ml of 1% lidocaine plus 1 ml of epinephrine 1:1,000 plus 1 l of normal saline). We used the Coleman microcannula technique through two small (3 mm) abdominal incisions to harvest fat tissue [3], obtaining 50 ml of lipoaspirate.

**Fig. 1** Case report. (A) adherent and painful scar at right shoulder. (B-C) At 1 year after treatment, skin was pliable and well separated from the underling fascia



## Fat Processing

Lipoaspirate was centrifugated (3,000 rpm for 3 min), obtaining 30 ml of fatty tissue. Refined fat then was transferred into a 1- or 3-ml Luer-Lok syringe.

## APRP Processing

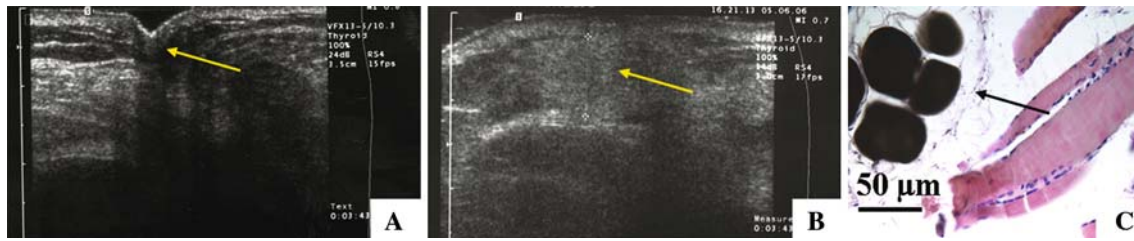
A self-contained disposable kit (Cascade Medical FIBRINET System, Wayne, New Jersey) was used to process 20 ml of peripheral blood. The kit consists of two or more sterile evacuated blood collection tubes, needles, and a transfer device. The Cascade Medical FIBRINET System is designed to be used for the safe and rapid preparation of autologous platelet-rich plasma from a small sample of blood. The whole blood was centrifuged at 1,000 g for 15 min.

## Implantation of Fat Mixed With APRP

The refined fat was mixed with the APRP. An adipose-platelet gel then was obtained and injected under the scar (Fig. 3A). A small amount of gel was used for histologic studies. Tissues were fixed in 10% formalin for 24 h at 4°C, dehydrated with a graded alcohol and xylene series, and embedded in paraffin. Then 8- $\mu$ m sections were cut with a Reichert Jung rotary microtome, mounted on glass slides, and stained with hematoxylin and eosin. Histologic studies demonstrated fat integrity in the APRP context (Fig. 3B).

## Postoperative Follow-up Evaluation

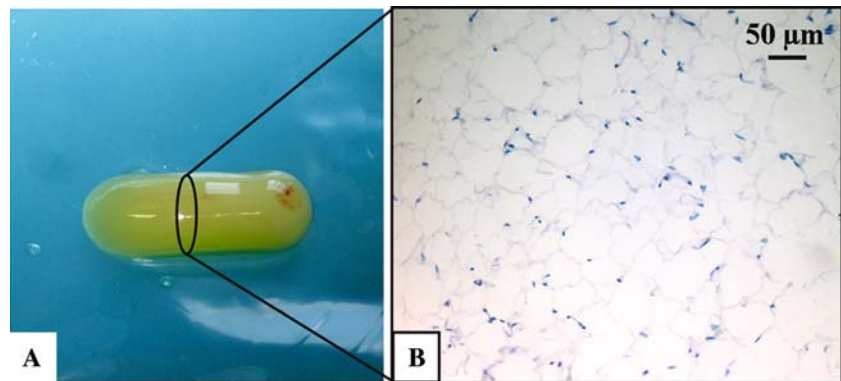
The postoperative course was uneventful, and the early postoperative result was very satisfactory to both the surgeon and the patient. In particular, the patient reported complete remission of pain at the shoulder. Ultrasonography performed after 6 months and 1 year showed maintenance of adipose tissue (Fig. 2B). The skin was pliable and well separated from the underling fascia



**Fig. 2** Ultrasonography and histologic analysis. (A) Ultrasonography shows an incisure created by scar tissue (yellow arrow). (B) At 1 year after surgery, ultrasonography shows connective tissue between the

skin and fascia (yellow arrow). (C) Histologic analysis confirms the presence of adipose tissue between skin and muscle tissue (black arrow)

**Fig. 3** Fat mixed with autologous platelet-rich plasma (APRP). (A) Macroscopic aspect of the APRP gel mixed with fat tissue. (B) Hematoxylin and eosin staining adipocytes inside the gel



(Fig. 1C). We performed histologic analysis after 1 year to confirm survival of adipose tissue. We used a 4-mm biopsy punch to harvest a small amount of tissue in the treated area. Specific staining of adipose tissue (OsO<sub>4</sub>) for histologic analysis showed healthy adipocytes near muscular tissue (Fig. 2C).

## Discussion

The objective for this case report was to describe a completely autologous novel technique for *in vivo* adipose tissue engineering in which autologous fat is included in APRP and injected as a gel into a subcutaneous pocket. Our aim for the case report was only to stimulate future research for confirmation of reported results similar to previous preliminary reports using other biologic gel [8, 11]. It was not our purpose to compare this technique with other surgical techniques, but rather to propose a first step in creating an *in vivo* tissue-engineering approach to adipose tissue. This tissue-engineering approach is intended to preserve the delicate structure of adipocytes and provide an ideal environment rich in growth factors for their survival and for early recruitment of new capillaries inside the construct.

Moreover, recent studies have demonstrated that the stromal-vascular cell fraction of adipose tissue represents a rich reservoir of regenerative precursor cells that have

proangiogenic capabilities comparable with those of bone marrow-derived stem cells [7]. The need to preserve this delicate cellular component is evident. Coleman [2] stated that the most important consideration in fat grafting is the method of placement.

What do we need to create an adipose tissue construct of significant volume? Results of numerous studies after an *in vivo* tissue-engineering approach suggest two fundamental points: (1) an optimal microenvironment that allows correct architectural adipocyte distribution, cell interaction, growth, and differentiation, and that offers early protection from surrounding inflammatory events, and, as soon as possible, (2) the arrival of a microcapillary network that delivers the proper nutrient and oxygen levels to injected cells [1, 4, 5, 9, 12]. As a response to these necessities, the more promising approaches are flap prefabrication, aimed ultimately at generating vascularized tissues, and the creation of “intelligent” biomaterials capable of mimicking the natural *in vivo* environment rich in cell signals.

This preliminary observation established that APRP can support an adipose tissue graft, preserving its volume for 1 year. We set the last follow-up point after 1 year because, according to the literature, fat that survives the inflammatory processes and is present at 1 year appears to be stable indefinitely. As a three-dimensional gel that can be mixed easily with cellular components, APRP provides an autologous environment rich in growth factors secreted by

platelets. It is well demonstrated that these growth factors (platelet-derived angiogenesis factor, vascular endothelial growth factor, epidermal growth factor, platelet-derived endothelial growth factor) stimulate endothelial cells near the APRP application site to proliferate and form new capillaries that extend into the gel [3]. This could begin the process of angiogenesis, facilitating early contact of implanted adipocytes with a new microcapillary bed.

Platelets also secrete other proteins such as fibrinogen, vitronectin, and fibronectin, which play a critical role in the regulation of cell–cell interactions and cell spatial organization [3]. It seems that APRP offers a good initial environment for adipocyte survival and, moreover, that it is a completely autologous biodegradable gel. The preparation of APRP gel must be done at the time of surgery, using a sterile disposable kit, in the operating room. The obtained gel could be mixed rapidly with the desired cellular components, allowing the creation of a cellularized gel for surgical use.

The proposed approach satisfies many safety considerations. Cells (adipocytes) are completely autologous and immediately employed without any type of *in vitro* preconditioning. Biomaterials are obtained from an autologous peripheral blood sample processed without any media components. Finally, the surgeon mixes adipocytes with the APRP gel in the operating room using dedicated and sterile instruments. In addition, the use of autologous cells and autologous biomaterials does not require collection of effectiveness data. Effectiveness was confirmed by histologic and ultrasonographic analysis that showed fat survival 1 year after implantation.

The reported case is the preliminary result of a long-term project involving the development of a completely autologous adipose tissue construct. Obviously, procedures will need to be optimized to increase the survival of adipocytes *in vivo*, particularly when clinically significant amounts of adipose tissue need to be reconstructed, making revascularization an essential process. In our opinion, a single-step approach may not be successful for cases in which a large adipose tissue defect needs to be treated. We

are confident that if this easy-to-handle approach can be fine-tuned and combined with more innovative regenerative medicine, the reconstruction of adipose tissue—that long-standing dream of plastic surgeons—will be a clinical option in the not-too-distant future.

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