ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

The synergy between lasers and adipose tissues surgery in cervicofacial rejuvenation: histopathological aspects

ANDREEA CRISTINA NIŢĂ[1)], DANA MIHAELA JIANU[2)], I. P. FLORESCU[3)], MARIA FILIPESCU[2)], O. COBANI[2)], S. A. JIANU[4)], D. A. CHIRIŢĂ[5)], ADRIANA BOLD[6)]

[1)]*Department of Dermatology*
[2)]*Department of Aesthetic Surgery*
ProEstetica Medical Center, Bucharest
[3)]*Department of Plastic and Reconstructive Surgery, "Bagdasar–Arseni" Emergency Hospital, Bucharest*
[4)]*Department of General Surgery, ProEstetica Medical Center, Bucharest*
[5)]*Department of Dermatopathology, Domina Sana Medical Center, Bucharest*
[6)]*Department of Histology, University of Medicine and Pharmacy of Craiova*

Abstract

Background and Aims: Nowadays patients want a long-lasting youthful appearance but through a less invasive approach. Our unique approach, "AdipoLASER reJuvenation (ALJ)", involves a variety of less invasive techniques based on simultaneous laser resurfacing, and lipolysis together with adipose tissue graft and redistribution. Recently, we added to this platelet-rich plasma therapy. We conducted a study aiming to emphasize the histopathological changes occurred following these procedures. *Patients and Methods*: Between 2011–2012, we included 50 patients that were preparing for abdominoplasty (tummy tuck) in which we applied ALJ procedures, in two comparative zones of inferior abdomen. Histological examination varied from 10 days to four months, according to the time of the abdominoplasty. *Results*: There was a notable histological difference between stimulated and non-stimulated fat graft regarding adipose cells structure and number, neocollagenesis, and dermal matrix remodeling. *Conclusions*: The low level laser therapy effect (LLLT) of the fractional CO_2 laser combined with the effect of the growth factors derived from activated platelets (PRP) prolonged the life and improved the take of the facial fat graft, increase collagen formation and lead to a better remodeling of the dermal matrix. This unique surgical combination of all four approaches in our ALJ with additional PRP offers a real less invasive but strongly visible – yet natural result – as an alternative to the classic facelift.

Keywords: surgical lasers, fat graft, LLLT effect, preadipocytes, histopathological aspects.

Introduction

At the present, plastic surgery has to face some new facts: the worldwide graying of population, the increase of public demand for looking younger and better and a global tendency for less aggressive procedures as shown in a survey issued by an *American Society for Aesthetic Plastic Surgery (ASAPS) 2010 Survey*. This survey concluded that worldwide, only 2.27% from the interviewed patients were operated for facelift (rhytidectomy). However, 4.51% of the very larger remaining group was being treated with autologous fat, and 1.02% with laser resurfacing. In other words, there were 5.53%, almost 2.4 times larger the number of patients who elected to have a fat transposition procedure and laser resurfacing instead of classical facelift. The possible reason for this growing global public reluctance for a full rhytidectomy could be the severity of the sequels after this kind of surgery.

To meet the growing demand for a less invasive but effective and long-lasting surgical alternative to conventional rhytidectomy, we developed an original combination, a new surgical approach based on the synergy between fat graft, laser procedures (laser-assisted lipolysis, fractional laser CO_2), named by us "AdipoLASER reJuvenation" or ALJ, on which we recently added growth factors derived from platelet-rich plasma (PRP). This combined therapy could even address issues that the standard rhytidectomy is not able to do, such as improvement of overall skin tone and condition.

Autologous fat transplantation (fat grafting) is a well-established technique in surgery. The most significant problem of this method is the unpredictable resorption rate. Many studies have been done on the subject and the techniques have evolved to produce maximum tissue retention at the transplanted site [1]. The discovery of preadipocytes, their mesenchymal origin and their role as pluripotent stem cells have been used in regenerative medicine to maintain the graft tissue, due to their capability to differentiate, to stimulate the angiogenesis and collagen synthesis [2, 3].

ISSN (print) 1220–0522 ISSN (on-line) 2066–8279

Based on the clinical results we published in 2012, 63.2% excellent patients' overall satisfaction rate after ALJ [4], we want to enhance the role of laser and PRP in fat graft survival in our technique with histopathological evidence. Because all of the patients in which we applied ALJ addressed for face rejuvenation, it was impossible to obtain tissue for histopathological examination, so we decided to obtain data from patients scheduled for abdominoplasty.

Patients and Methods

Based on the ProEstetica Medical Center (Bucharest, Romania) Research Ethics Committee – Institutional Review Board – Resolution 7/2011, the study was conducted from 2011 to 2012, and 50 patients were included. Inclusion criteria were patients older than 18 years preparing for abdominoplasty. All patients gave informed consent, their full medical history and their skin type ranged between III and IV on Fitzpatrick scale.

At each patient, two separate areas (A, B) were marked on the apron of skin and fat, which it was removed after the abdominoplasty. On the A area, we injected only the fat; on the B area, we stimulated the injected fat graft with fractional CO_2 laser and activated PRP. The skin biopsies were taken during abdominoplasty, so the histopathology cups' harvest times were different respectively from 10 days to four months.

The procedure for the fat harvesting and lipofilling was identical with what we used in our ALJ as follows:

- marking the donor and treatment sites;
- harvesting fat from the flanks or the abdomen with a 10, 20 or 60 cc syringe attached to a 14 or 16 G needle;
- when using a 14 G needle, 60–80 cc of fat should be extracted;
- allow the fat to stand for a moment, discard the lower fraction (water and blood) followed by gentle manual centrifugation for three minutes;
- perform fat face transplantation to the marked areas with a 1 cc syringe and a Fischer cannula Φ 1.2–1.4, placing small droplets of fat in the tissue following Coleman's lipostructure technique [5].

For resurfacing, we used a fractional carbon dioxide (CO_2) (λ=10.600) (MedArt 610 FRx, ASAH MEDICIO A/S, Valseholmen 11–13, Denmark) at the following parameters: power 9–12 W, time 5 ms, medium density. The laser parameters were matched with individual Fitzpatrick's type.

For activated PRP, we used a standard PRP kit GLOFIN (Salo, Finnland) – from 8.5 cc patient' blood up to 2 cc PRP it was obtain after two centrifugations. The induction is triggered by addition of calcium chloride. It was injected in the mid and deep dermis. During the initial burst of activity within the first hour, about 95% of the pre-synthesized growth factors are released, and during the remaining seven days of their viability, the platelets synthesize and secrete additional growth factors [6].

Tissue fragments were processed using Hematoxylin–Eosin staining and immunohistochemical labeling with mouse antigen DLK1. Comparisons were made in terms of fat cells (quantitative and qualitative), fibroblasts and blood vessels at these levels, using different magnifications (50×, 100×, 400×).

Results

Histopathological examination of the stimulated areas compared to the non-stimulated one showed marked differences in terms of young adipocytes presence, pre-adipocytes, local cells growth, neovascularization and dermal matrix remodeling in all of the cases.

At the interface between reticular dermis and the subcutaneous tissue, we found a richer cellularity with frequent fibroblasts and fibroblast-like cells on the stimulated one, compared with the inflammatory infiltrate with numerous lymphocytes, plasmocytes, mastocytes and some few fibroblasts found on the non-stimulated one (Figure 1). Also, a large amount of extravasated erythrocytes were presented in the adipocytes' lobules on the non-stimulated area, which had been phagocytized by siderophages on stimulated one (Figure 2). So, a more rapid healing process was noticed in the stimulated area compared with non-stimulated one in the earlier harvest cups.

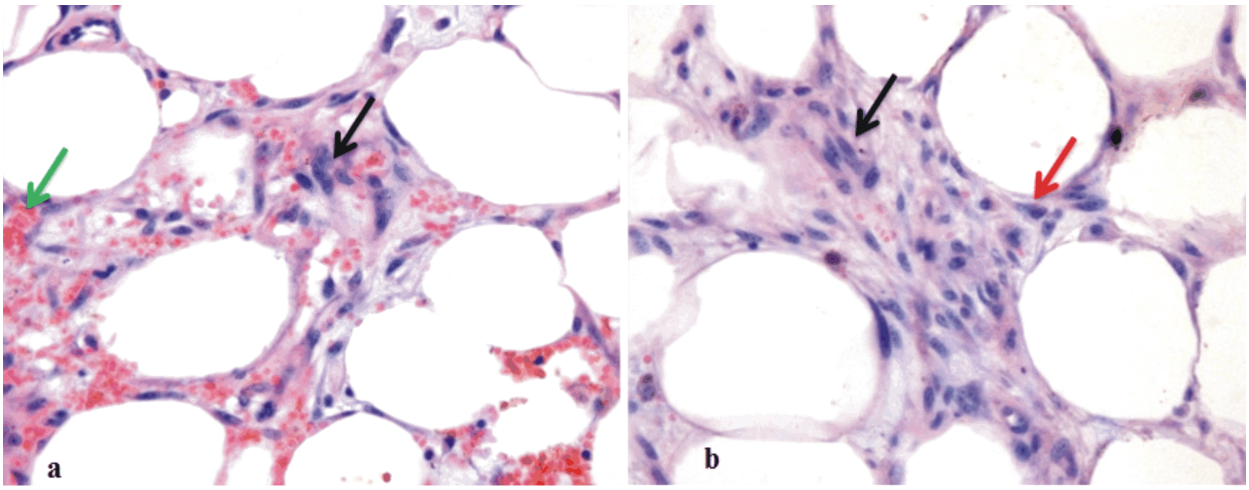

Figure 1 – *Interface between dermis and subcutaneous tissue, 10 days: (a) Non-stimulated; (b) Stimulated graft. HE staining, 400×.*

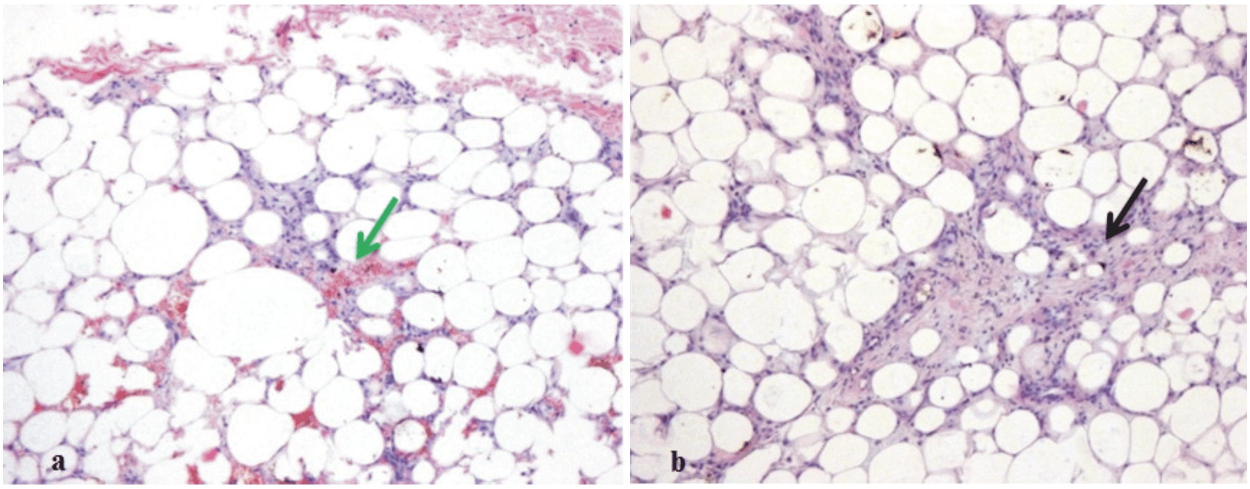

Figure 2 – ***Healing phase, 10 days: (a) Non-stimulated; (b) Stimulated graft. HE staining, 400×.***

The adipocytes were smaller and round with increased vascularization, large septa, fewer cysts and vacuoles, and a richer cellularity with numerous fibroblasts surrounded by more organized young collagen fibers in the stimulated area compared with non-stimulated one (Figures 3 and 4). We also noticed the presence of pre-adipocytes (immunohistochemical label with mouse antigen DLK1) in all of our stimulated area up to three months (Figure 5).

Early the harvest cup was made more preadipocytes were found, as time passes the stimulated area shows increased density of extracellular matrix surrounding the fatty tissue and between the dermis and the hypodermis (Table 1).

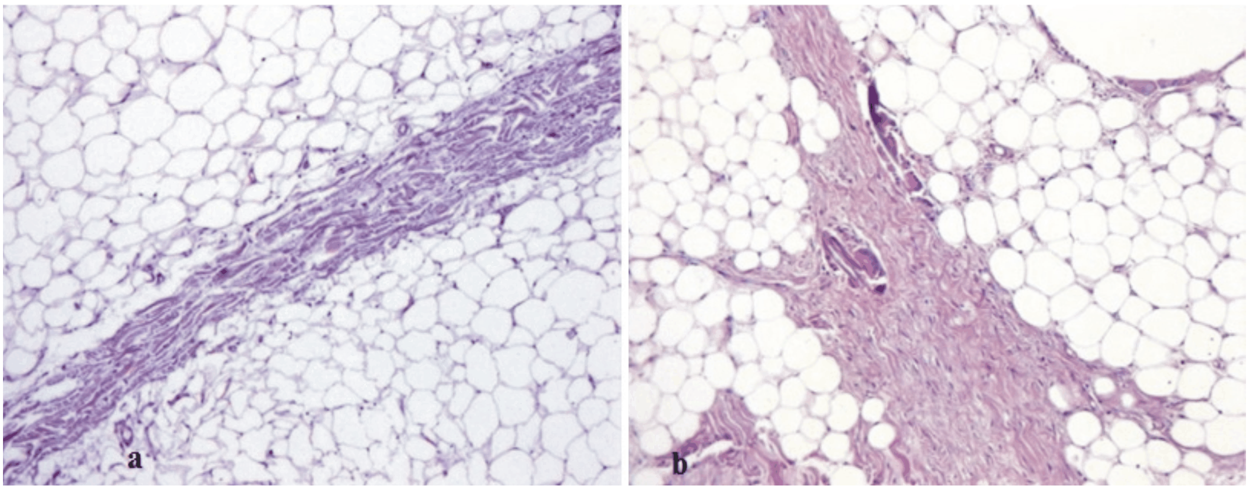

Figure 3 – ***Extracellular matrix surrounding adipose lobules, three months: (a) Non-stimulated; (b) Stimulated graft. HE staining, 100×.***

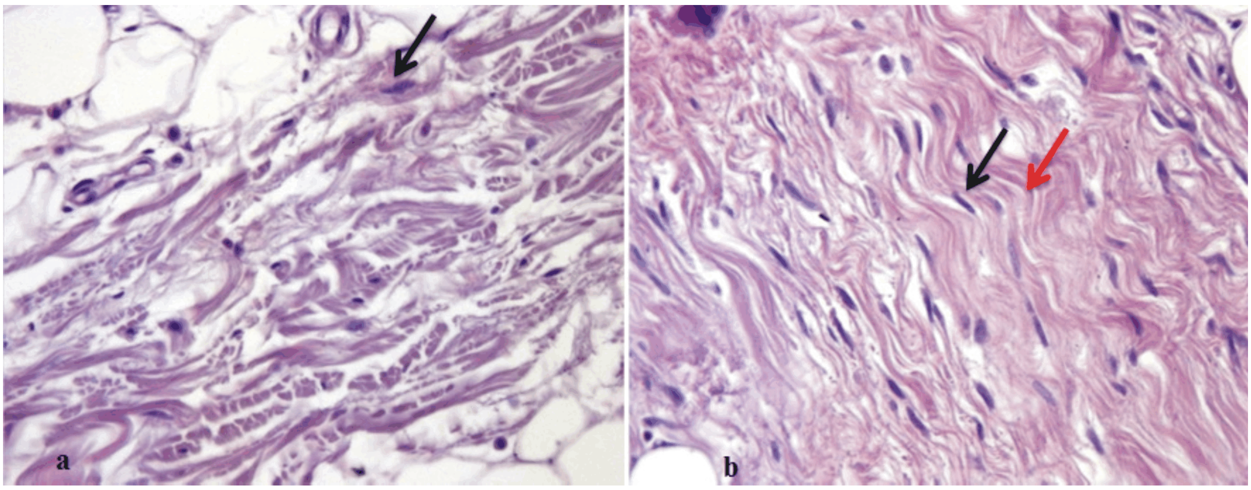

Figure 4 – ***Interface between dermis and subcutaneous tissue, three months: (a) Non-stimulated; (b) Stimulated graft. HE staining, 400×.***

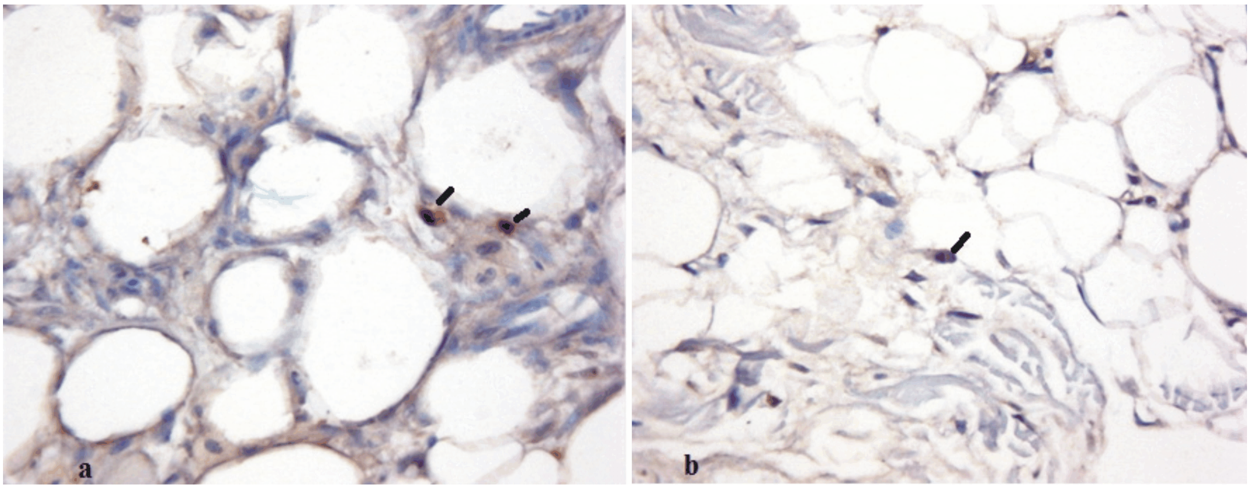

Figure 5 – ***Preadipocytes on stimulated graft: (a) 10 days; (b) Three months. Immunoperoxidase staining DLK1, 1:500, 400×.***

Table 1 – ***Histological modification on stimulated fat graft as time passes***

	10 days	Three months	Four months
No. of fibroblasts	+	++	+++
Dermal matrix remodeling	+	++	++++
Presence of preadipocytes	+++	++	0

Discussion

Each component of our four ALJ approach has its own advantages when used alone, but when used in combination we found that the synergic effect gives a superior result compared to the individual components. Fat grafting is a well-established method for contour correction in areas of the face with volume loss due to fat resorption and slippage during the aging process. However, persuading the graft to survive and maintain the good effect can be problematic. In this context, we constantly need to think for new methods of improvement.

Conventional surgical lasers produce photothermal and other effects in tissue, which are destructive in nature: there is a permanent change in the laser irradiated tissue like incision, vaporization and coagulation. The reaction in tissue following such laser irradiation is therefore above the survival threshold of normal tissue, causing death or disruption, classed as high-level laser treatment (HLLT). However, in the same time any surgical lasers also stimulate the tissues especially through non-thermal, photobioactivation and these are classified as Low Level Laser Therapy (LLLT) effect [7].

HLLT occurs at temperatures from above 40^0C to 200^0C and, depending on the temperature, can achieve carbonization, vaporization, coagulation, denaturation and degradation of proteins. In our study, the major photo-surgical effects are coagulation and protein degradation, which are sufficient to induce the wound healing process.

LLLT, on the other hand, works at temperatures under 40^0C (or with no temperature rise at all) inducing photo-bioactivation without any damage. The resulting photo-chemical, photodynamic, photoenzymatic, and photo-immune effects act directly on cells – at a subcellular organelle level – leading to a variety of results including cell repair, enhancement of cellular function and cellular proliferation. It is well accepted that the energy of photons – when absorbed directly in cells or tissue during the LLLT process – may affect cellular metabolism and signaling pathways. Reported results include increased cell proliferation and migration (particularly by fibroblasts), increased tissue oxygenation, modulation in the levels of cytokines, growth factors and inflammatory mediators [8].

The macroablative columns (MACs) associated with ablative CO_2 fractional resurfacing induce wound healing, with elastinogenesis and neocollagenesis in the upper and mid dermal layers, resulting in a much better-organized dermal matrix and younger-looking epidermis [9, 10]. They also have an associated zone of low photon intensity surrounding the damaged tissues, thereby assisting with acceleration of the wound healing process and also photo-bioactivation of the transplanted fat cells to ensure better graft take with less initial resorption. In this case, we can say that CO_2 laser is a non-contact LLLT device for our fat graft.

Based on the recent data released by Marx RE (2004) [11] and Cervelli V *et al.* (2009) [12] on the role of platelet growth released factors in accelerating healing entitled us to assign in this technique [13]. The immediate effect was to shorten healing time (peeling) post-laser from seven days to four days and possible to increase graft survival and promote the proliferation of human adipose-derived stem cells. Although several mechanisms may be responsible for this result, the most important mediator of the survival of fat grafts is induction of angiogenesis. PRP contains mitogenic and chemotactic growth factors important in angiogenesis, including VEGF and EGF [14]. The histological evaluation indicated that the stimulated one (with PRP and laser) had a more highly vascularization and a fewer cysts and vacuoles which may be attributed to inadequate vascularization in the non-stimulated one. However, further studies both *in vitro* and *in vivo* are needed to see if mixing these factors with adipose tissue increases survival of regenerative cells. In the present study were administered separately, due to the assumptions remaining that blood macrophages could destroy the transplanted fat cells [15].

Conclusions

The global tendency in cervicofacial rejuvenation is showing a greater demand for fat graft and laser procedures and less demand for the classical surgical facelift. Our "AdipoLASER reJuvenation" approach is a personal complex method, a simultaneous combination of four surgical techniques with standard microliposuction and laser-assisted lipolysis as the reductive techniques and fat grafting and full-face fractional CO_2 laser resurfacing as the regenerative techniques, but all embodied in a single session. We consider that this specific and customized combination is the key in providing best individual solutions to each patient. The LLLT component of the lasers we used and growth factors from PRP are possible optimizing factors for the fat graft survival, offering new opportunities for research and clinical applications. From this "closed" less invasive surgical approach could benefit patients seeking to improve their appearance and quality of life, patients with contractile and atrophic scars, HIV+ patients on HAART therapy with atrophic lipodystrophy. Further studies with much larger population are warranted to confirm the result of this preliminary study.

References

[1] Coleman SR, Update on structural fat grafting. In: Coleman SR, Mazzolla RF (eds), *Fat injection: from filling to regeneration*, Quality Medical Publishing, St. Louis, MO, 2008, 93–203.

[2] Zhu M, Zhou Z, Chen Y, Schreiber R, Ransom JT, Fraser JK, Hedrick MH, Pinkernell K, Kuo HC, *Supplementation of fat grafts with adipose-derived regenerative cells improves long-term graft retention*, Ann Plast Surg, 2010, 64(2):222–228.

[3] Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH, *Multilineage cells from human adipose tissue: implications for cell-based therapies*, Tissue Eng, 2001, 7(2):211–228.

[4] Jianu DM, Filipescu M, Jianu SA, Nita AC, Chirita DA, *The synergy between lasers and adipose surgery in face and neck rejuvenation: a new approach from a personal experience*, Laser Ther, 2012, 21(3):215–222.

[5] Coleman SR, *Facial recontouring with lipostructure*, Clin Plast Surg, 1997, 24(2):347–367.

[6] Pietrzak WS, Eppley BL, *Platelet rich plasma: biology and new technology*, J Craniofac Surg, 2005, 16(6):1043–1054.

[7] Ohshiro T, *Low reactive level LASER therapy: practical application*, John Wiley & Sons Inc., 1991, 6–76.

[8] Ohshiro T, *New classification for single-system light treatment*, Laser Ther, 2011, 20(1):11–15.

[9] Laubach HJ, Tannous Z, Anderson RR, Manstein D, *Skin responses to fractional photothermolysis*, Lasers Surg Med, 2006, 38(2):142–149.

[10] Kauvar ANB, Warycha MA, Wrinkles and acne scars: fractional ablative lasers. In: Raulin C, Karsai S (eds), *Laser and IPL technology in dermatology and aesthetic medicine*, Springer-Verlag, Berlin–Heidelberg, 2011, 307–318.

[11] Marx RE, *Platelet-rich plasma: evidence to support its use*, J Oral Maxillofac Surg, 2004, 62(4):489–496.

[12] Cervelli V, Gentile P, Scioli MG, Grimaldi M, Casciani CU, Spagnoli LG, Orlandi A, *Application of platelet-rich plasma in plastic surgery: clinical and in vitro evaluation*, Tissue Eng Part C Methods, 2009, 15(4):625–634.

[13] Kakudo N, Minakata T, Mitsui T, Kushida S, Notodihardjo FZ, Kusumoto K, *Proliferation-promoting effect of platelet-rich plasma on human adipose-derived stem cells and human dermal fibroblasts*, Plast Reconstr Surg, 2008, 122(5):1352–1360.

[14] Eppley BL, Woodell JE, Higgins J, *Platelet quantification and growth factor analysis from platelet-rich plasma: implications for wound healing*, Plast Reconstr Surg, 2004, 114(6):1502–1508.

[15] Sommer B, Sattler G, *Current concepts of fat graft survival: histology of aspirated adipose tissue and review of the literature*, Dermatol Surg, 2000, 26(12):1159–1166.

Corresponding author

Dana Mihaela Jianu, Associate Professor, MD, PhD, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Department of Aesthetic Surgery, ProEstetica Medical Center, 38–40 Tudor Ştefan Street, 011658 Bucharest, Romania; Phone/Fax +4021–230 52 00, Mobile +40722–208 071, e-mail: djianu02@gmail.com

Received: May 12, 2013

Accepted: November 8, 2013