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original article

Processing techniques of the adipose tissue: centrifugation *vs* decantation

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SUMMARY: **Processing techniques of the adipose tissue: centrifugation *vs* decantation.**

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The processing techniques of the adipose tissue represents one of the most debated topics. There are different processing techniques. As of today, the standard considered procedure is the centrifugaton according to Coleman. In addition, other procedures include decantation/sedimentation, filtration and water jet force, which offer the specialist valid alternatives. In the present review, the clinical evidence of the techniques of centrifugation and decantation will be evaluated by studying histological data; maintenance of tropism and the maintenance of mesenchymal cells. These two aspects are different in the centrifugation and decantation techniques.

KEY WORDS: Adipose tissue - Processing techniques - Centrifugation - Decantation - Sedimentation - Mesenchymal cells.

Introduction

In the international literature of medicine and regenerative surgery, alongside a progressive proposal of new clinical applications and growing experimentations, one of the majorly debated topics focuses on the modality of withdrawal and processing of the fat, the common feeling of the researches and the surgeons indicate the necessity to obtain an adipose tissue rich of vital regenerative cells, not damaged from the withdrawal and processing systems.

As a result of an increasing number of grafting procedures of adipose tissues, different laboratories have developed their own methods for the processing of fat, like the methods of closed collection, centrifugation, water jet force and other filtration devices, or even simple techniques of decantation (1). The adipose tissue is rich of mesenchymal stem cells able to promote the spontaneous healing of the tissues. The main sources of mesenchymal stem cells are the adipose tissues, the peripheral blood, the ombelical cord and the bone marrow. Compared to all the other withdrawal sites, the adipose tissue has a greater facility of access, less invasive and has a high concentration of mesenchymal stem cells, therefore, has a great regenerative potential.

The recent studies which analyse the treatment of adipose cells prior the transplant, analyse the cellular's qualitative and quantitative evidence after the centrifugation or decantation. From the end of the eighteen-hundreds, clinical experiences of replanting the adipose tissue for filling purposes have been reported. Since then, the procedure has notably diffused for aesthetic purposes – both reconstructive and functional fillers. Doctor Coleman introduced the technique of lipostructure with centrifugation as a pivotal treatment. In the last decade, controversies have rose on the influence of the techniques of withdrawal, processing and fat injections on the graft of adipose tissue.

This review's scope is to analyse these two techniques and understand which is the most adequate for the specialist's requests. Recent studies do not reveal the objective differences, instead each of these

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techniques is considered first choice in relation to the necessity and the specialist's ability (2).

Although these techniques present advantages, as of today the centrifugation is considered the gold standard. The motive for such choice seems to be affected more by the practice of the specialist rather than the objective results. Despite this, opinions remain to conflict. There are indeed studies in which the qualitative efficiency of the decantation in compared to centrifugation is evident (3). The present study analyzes the histological differences of the regenerative cells obtained through the centrifugation and decantation, in particular the maintenance of cell trophism and the major number of mesenchymal cells. These two aspects result different in the centrifugation and in the decantation.

Centrifugation

It has been the most used method in the last three decades used to separate the fat from the other components of the tissue. According to some studies, the use of centrifugation continues to be the gold standard for the preparation of the autologous transplant of adipose tissue (lipofilling) (4).

Figure 1 - Close-up photograph of the four distinct layers issued from the centrifuged lipoaspirate: the superior, middle, and inferior layers and pellet (2).

Through centrifugation, the separation of the cells in layers is obtained: the superior layer is a fraction of oil and lipids determined to break the cell membranes of the traumatised adipose cells; the intermediate layer contains the adipose tissue; the inferior layer is formed essentially of blood cells (Figure 1).

According to studies which have detailed the exact number of rounds and the right speed of centrifugation, the use of centrifugation is optimal to obtain a good density of the adipose tissue with a significative number of progenitor cells and a good removal of excess liquid. The right speed and duration identified is 1300 gpm to maintain for 5 minutes. At greater speeds the results appeared qualitatively insufficient (4, 5). Some Authors also recommend to associate a treatment WAL (Water Jet Force) lipoaspirates before grafting, in which have registered high percentages of CD35+/CD45- with low levels of apoptosis, better engraftment and optimal angiogenesis (5). Nonetheless, other Authors have found a plentiful presence of endothelial cells and of mesenchymal stem cells (MSCs) within the deep layers of the centrifugal even without the WAT (6). Instead other studies show a sharp reduction of MSCs with parallel decrease of intact adipocytes, comparing the centrifugation to the results shown of the decantation in which a major vital number of MScs remain present (7). Therefore, the literature shows that centrifugation is confirmed to be an optimal processing technique which can be improved further with adroitness of the surgeon, for example through the WAT and the reduction of speed of the centrifugal. Moreover, it has been observed that the deep layer of the centrifugal contains adipocytes more vital together with the stromal cells of the vascular fraction which brings a better engraftment. The cells compromised are distributed in the superior layer of the centrifugal, bringing a reduced retention of volume. Although with a lower content stromal of cells of the vascular fraction, the intermediate layer owns a considerable number of intact adipocytes which are capable of maintaining a partial volume after the implantation, suggesting that the intermediate layer represents an alternative source of fat when necessary great volumes of adipose grafts are (6).

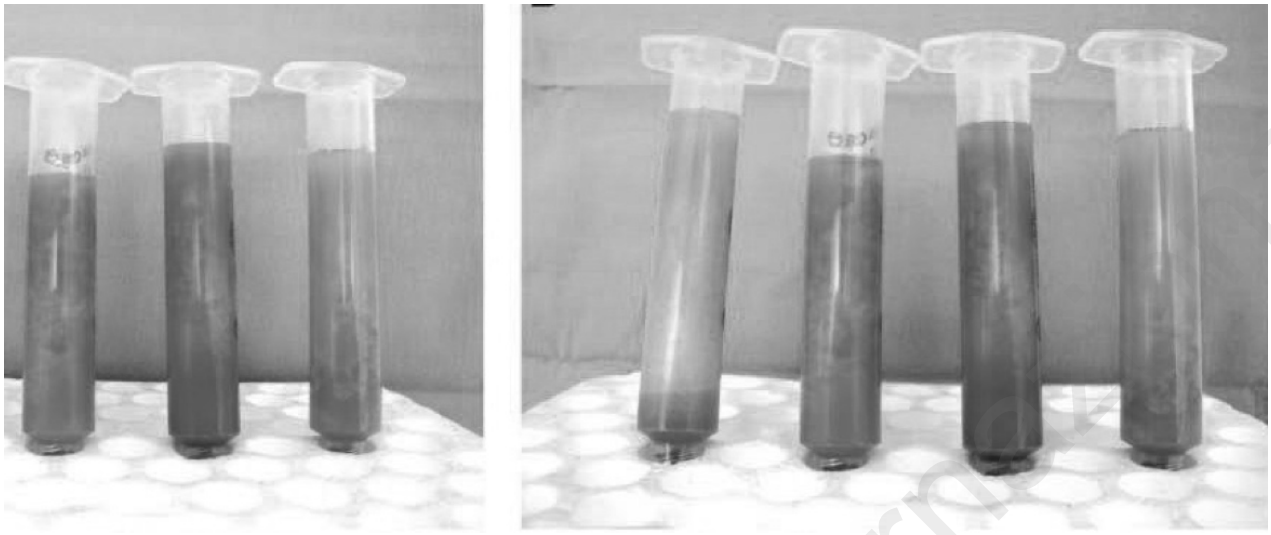

Figure 2 - Decanted lipoaspirate in 10-mL syringes, showing the three distinct layers (8).

Decantation

A more recent school of thought proposes decantation as a new pre-transplant processing method of the adipose tissue (Figure 2). A high count of whole cells after decantation is shown from the studies (8). To this day different methods of decantation exists, from the simple sedimentation in syringe, to the simple water jet force or through special devices (i.e. Cytoi Pure Graft with lactate ringer solution), to the membrane filtration, which through the physical separation enables only the vital adipose tissues and the growth factors to concentrate in the syringe (9). A very recent study describes the necessity of a brief timing between the time of withdrawal, processing and the injection of lipoaspirates. Apoptosis and the mortality of adipocytes are directly proportional to the time of decantation. For these reasons the Authors suggest the timing of execution between the processing and the injection to be brief as possible (10). Different Authors support the superiority of the processing technique through decantation based on the comparisons between the histological layers of adipose tissue decantated and centrifuged. Indeed, from the histological sections of adipose tissue aspirated post-decantation and post-centrifugation, the sample decantated (Figure 3A) shows nucleated adipocytes relatively intact with normal morphology (arrow Figure 3). The lipoaspirates sample centrifuged (Figure 3B) instead has a smaller number of intact adipocytes and mostly traumatised (arrow Figure 3B). The graphic (Figure 3C) shows the number of adipocyte cells intact in a determinate section of the histologycal area with an average ± standard derivation of four patients, where $P < 0,05$ between all the samples. Dimensions of the bar: 200 μm.

Table 1 represents the number of the fraction of hematopoietic cells (CD45+) and non-hematopoietic (CD45-) in decanted aspirates. It is in fact an evident presence of the hematopoietic fraction in the withdrawal.

Discussion

The processing systems of adipose tissue destined to the autologous transplant represent one of the most controversial topics in the clinical practice and in the international literature.

Centrifugation is the standard considered procedure according to Coleman, whose criticism rose from literature studies seems to be essentially bound to the speeds of the rounds per minute. The ideal speed to obtain a better population of regenerative cells seems to be between 1200 and 1300 gpm. In clinical practice, especially that for aesthetic purposes, the usage of alternative processing techniques are growing, including decantation, filtration and water jet force of the aspirated adipose tissue. The centrifugation represents the choice of elections of

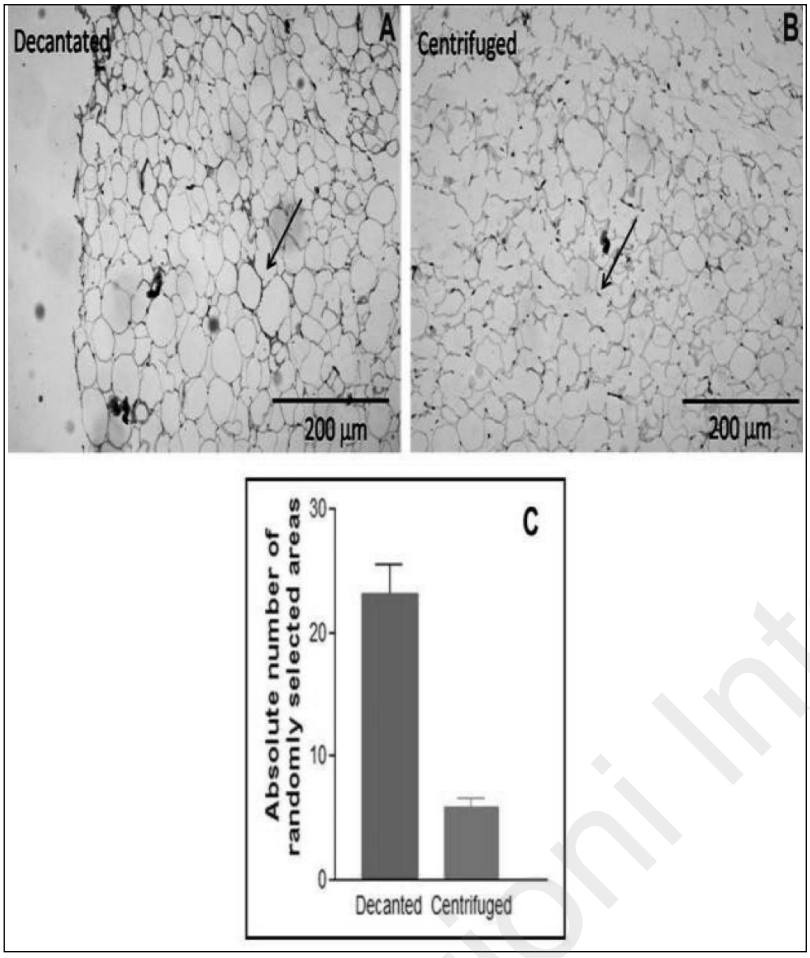

Figure 3 - Histologic sections of aspirated adipose tissue processed by decantation and centrifugation. (A) The decanted lipoaspirate sample shows relatively intact, nucleated adipocytes (arrow) and overall normal morphology. (B) The centrifuged lipoaspirate sample has a small number of intact adipocytes (arrow) and more extensive trauma. The adipocyte count was performed based on morphology observation and represents only intact cells. (C) A graph depicting the mean ± standard derivation of four patients, where P < .05 among all samples. Bar size: 200 µm.

TABLE 1 - COMPARISON ON THE COMPOSITION AND VIABILITY OF ADIPOSE TISSUE ASPIRATED AND PROCESSED FOR LIPOFILLING BY DECANTATION AND CENTRIFUGATION (4).

	Decanted	Centrifuged	Pellet
CD45+	6.2 ± 2.9	2.7 ± 1.6	8.8 ± 1.4
CD45- CD31+	3.8 ± 3.1	2.0 ± 0.7	7.1 ± 3.6
CD45- CD34+	2.8 ± 2.7	2.3 ± 1.7	4.3 ± 2.3

many specialists. A great part of their hospital departments possesses a centrifuge, which leads the team not to scour techniques like decantation, whose use is instead increasing in the non-hospital aesthetic sector. Therefore, it seems that the choice of processing through centrifugation is mainly bound by the habits of the specialist than the objective results as far as the registered results appear good for both techniques. In fact, from this study decantation emerges as the most rapid, most economic technique with an optimal maintenance of the adipose tissue's integrity, like instead from Table 1 there is a prevalence of the fraction of hematopoietic (CD45+) in the decanted withdrawal equal in respect to the centrifuge to witness a greater safeguard of the cell counts which then allows a better tissue engraftment. From the histological sections of lipoaspirates (Figure 3) post-decantation and post-centrifugation, the decanted sample (Figure 3A) shows nucleated adipocyte relatively intact with

good morphologists and optimal structural maintenance of the extracellular matrix. From the centrifuged sample (Figure 3B) to witness a more stressful processing and qualitatively and quantitatively inferior shows a reduction of adipocyte count and a modification of morphology. Figure 3C shows the number of adipocyte cells intact in a determinate section of histological area the advantage of the treatment with decantation which grants a greater cell count. To better the results of decantation some Authors have deepened the technique by adding for example or a water jet force through special devices (i.e. Cytoi Pure Graft with lactate ringer solution) or membrane filtration which through the physical separation, allowing it to concentrate only the vital adipose cells and the growth factors in the syringe to reduce the metabolic request by transplantation (9). To these precautions it has been added a very recent study which underlines the importance of the timing between the withdrawal, processing and time of injection of the lipoaspirates. Apoptosis and mortality of the adipocytes are directly proportional to the timing of decantation. For these reasons the Authors suggest a time of execution between the processing and injection to be as brief as possible (11). Instead, in the centrifugation there has been found a greater speed (gpm) to which it separes the lipoaspirates.

Conclusions

The decantation represents the technique which better preserves the structure of the extracellular matrix and the integrity of the mesenchymal cells (12) and which represents greater margins of improvement in respect to decantation. However, the differences between decantation and centrifugation are not sufficiently analyzed to define with determination the preferable of a technique in respect to the other due to lack of comparative studies with extensive case studies. Therefore further studies are necessary to settle the controversy.

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