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ORIGINAL ARTICLE

Breast reconstruction with autologous fat combined with platelet rich plasma: fighting between medical novelty and cancer biology

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Summary

The use of autologous fat transplantation for reconstruction purposes after mastectomy or Breast Conserving Treatment (BCT) for Breast Cancer (BC) has increased significantly the past twenty years. Adipose-derived stemm cells hold great tissue regenerative potential due to their established ability to improve the healing process through in situ differentiation and secretion of paracrine factors. Platelet-rich Plasma (PRP), contains high levels of diverse human growth factors for stem cells proliferation and differentiation in the course of tissue regeneration, and it has recently been accepted by many as a highly promising method for tissue regeneration. The molecular mechanisms mediating this effect are unclear and still remain under investigation. Major disadvantages on the use of PRP are not reported. Promising results in enhancing the survival of grafted fat has been shown with PRP with the potential of affecting patient's oncological outcome when applied on tumor excision sites.

Key words: *breast, cancer, reconstruction, autologous fat, platelet rich plasma, oncology*

Introduction

According to the American Cancer Society, the incidence of Breast Cancer (BC) amongst other kinds of neoplasia has been gradually increased during the recent past [1]. Nowadays the advances of reconstructive surgery allow the large number of younger patients affected by breast cancer to receive breast reconstruction after mastectomy. A number of approaches and modalities are now well established and available to the patients in the course of substituting the unavoidable loss [2].

In that direction, the use of autologous fat transplantation for reconstruction purposes after mastectomy or breast conserving treatment (BCT) for BC has increased significantly the past twenty years. Autologous fat consists of Adipose-Derived Stem Cells (ASCs), cells that hold great tissue regenerative potential due to their established ability to improve the healing process through in situ differentiation and secretion of paracrine factors [3].

However, to ensure the success of the regenerative mechanism, a wide variety of biochemical factors have been used to increase both the proliferation and the differentiation of stem cells when autologous cells are used. Platelet-Rich Plasma (PRP), contains high levels of diverse human growth factors for stem cells proliferation and differentiation in the course of tissue regeneration, and it has recently been accepted by many as a highly promising method for tissue regeneration [4].

On the other hand, the molecular mechanisms mediating this effect are unclear and still remain under investigation, however there are no reports of major disadvantages on the use of PRP and only a few relative contraindications were formulated

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[5]. PRP has shown promising results for enhancing the survival of grafted fat. It also should be considered that PRP theoretically has the potential for affecting patient's oncological outcome when applied on tumor excision sites [6].

Review methodology

A search of the PUBMED, Cochrane Library / Cochrane Register of Controlled Trials, and SCIRUS databases was conducted without language or date restrictions; updated again current as of date of publication, with systematic reviews and meta-analyses extracted separately.

Platelet Rich Plasma

Platelet Rich Plasma or PRP is made up of an amount of autologous human platelets in a small volume of plasma. The pathophysiology acting pathway of PRP is the loss of the cytoplasmic granules (release of their content); as a result, a-granules are converted to platelets [7]. Formation of a-granules includes seven of the main human growth factors. In these human growth factors the Vascular Endothelial Growth Factor (VEGF), two isomers of the Transforming Growth Factor (TGF-β1, TGF-β2) and three isomers of the Platelet-Derived Growth Factor (PDGF-αα, PDGF-αβ, PDGF-ββ) as well are formed [7,8].

The aforementioned human growth factors are then actively secreted from the alpha granules (a-granules), a process initiated along the procedure of blood clotting. The process begins ten minutes after blood clot is formed, and approximately 95% of the growth factors has been released an hour later [8]. As a result, PRP should be prepared in an anticoagulated cohort; following that, PRP must be applied to the desideratum site within ten minutes of clot formation [9]. This is the triggering act that leads to platelet formation; the newly synthetized platelets release additional molecules and proteins to amplify their surveillance for the following five to ten days.

Efficacy of Platelet Rich Plasma

Due to the PRP's composition out of human growth factors, PRP is known to favor the angiogenesis procedure as well as the differentiation of derived/stem cells through a variety of experimental protocols. Relating to angiogenesis, it has been proven that PRP stimulates endothelial cells at the application site, favoring both the proliferation and the formation of new capillaries as a result [7]. In addition, PRP triggers the proliferation of undifferentiated stem cells, amplifying the regeneration of the implicated tissue [10]. Undifferentiated stem cells migrate to the application site of the PRP where vascular growth factor is secreted, and it is at the site the proliferation of the stem cells is triggered at the presence of growth factor [11].

Adding to that, there have been reports of successful clinical application of PRP, in the management of large wounds, maxillofacial bone defects and to cosmetic surgery as well [12]. However, only a few of them examines the role of PRP determining the clinical outcome of its use. Most of these publications are case reports or case series, rendering their conclusions as questionable. Following that, there have been publications showing that PRP had doubtful effect when applied for graft enhancement [13].

This comes from the fact that PRP production technique is yet not standardized and low quality PRP can be produced by inadequate devices [8]. Little evidence exists regarding the proper PRP production and characterization [14].

There are no recommendations about the required increase of the platelets above the baseline in PRP. Some publications suggest that PRP should achieve a three- to eight- fold of increase of the baseline platelets [15]. Since the normal range of platelets on a healthy individual is from 150 to 400×10^3/mL (mean value=275×10^3/mL), an increase to a level of 775×10^3/mL for the platelets count is required. Regarding the production process, specimen centrifugation should be performed according to sterile standards, and a complete separation between platelets and red blood cells should be achieved, while platelets remain intact and undamaged in order to terminate the secretion of human growth factors along the platelets isolation procedure.

General and oncological safety of PRP

Considering the PRP's autologous preparation, it is inherently safe; it doesn't trigger an immune response and it doesn't transmit diseases such as HIV, hepatitis and others. Other than that, the safety profile of PRP administration for reconstruction in BC is yet to be confirmed. The first objections are coming from the fact that blood agar (into which produced PRP is contained) is vulnerable and prone to bacterial infections material. However, this danger can be ruled out because PRP is no different in formation compared to any other blood clot formed; as a result it cannot support bacterial growth more than any other blood clot [9].

Moreover, the most common method of platelet triggering is the addition of sodium chloride and thrombin to the PRP [16]. In the early 1990's, there

were reports correlating the development of antibovine antibodies (antibovine factor V) with the human clotting factors (cross reaction) in response to the use of the bovine product to provide hemostasis [17,18], resulting in a disorder of the coagulation process. However, the main question remains to be answered, is whether PRP is oncologically safe to be applied to sites where cancer has previously developed and then excised. This important question is wisely raised by many researchers.

PRP and mitogenicity

To date, the data on specifically PRP for breast reconstructive use in the oncology setting, as opposed to adipose-derived stem cells (ASCs) or autologous graft fating, is insufficient to determine its oncological safety, given the limitations of anecdotal and uncontrolled case study/series data [19]. As to underpowered preclinical data, this is inconsistent. Some studies suggest a endothelium-mitogenic adverse impact [20], while others suggest a comparably negative MSC (mesenchymal stem cells)-mitogenic impact, as on stromal stem cells [21] or dental stem cells [22].

A fly in the ointment

In addition, because of the ability of activated PRP to promote human ASCs and dermal fibroblast proliferation, primarily via the induction of high levels of PDGF-AB and TGF-β1 [23], problems and discrepancies occur: as the authors acknowledge, maximal cell proliferation was observed at 5% activated PRP levels, but at higher levels of 20%, unaccountably, no cell promotion occurred, recently published data decisively shows that the concentration of growth factors clearly influences the biologic effects of PRP on human mesenchymal stem cells, demonstrating a dose-dependent not absolute spectrum of biologic consequence based on the growth factor concentration involved [24]. Therefore, we must exert extreme caution in interpreting existing data. The vast variability in that the pro-mitogenic activity, depends on other co-resident factors such as secreted thrombospondin-1, where at 30% levels of PRP there was no promotion of cell proliferation, but rather the opposite; namely a dose-dependent inhibition of cell proliferation [25], in keeping with other findings [26].

What all the above suggests is that a number of well-designed studies is necessary. These future studies should examine whether higher levels of activated PRP are non-inferior in reconstructive outcome compared to lower levels, since this would bypass the threat of mitogenic impact yet preserve the application of PRP in breast reconstruction (and other related interventions).

PRP as anti-mitogenic?

Muddying the waters further, PRP can exert anti-inflammatory, COX2-inhibitory and CXCR4 gene expression-reductive effects in addition to Nuclear Factor Kappa B (NF-κB) inhibition [27], largely due to the mediation of hepatocyte growth factor (HGF), which collectively may have countervailing net anti-mitogenic activity.

PDGF alone may not be adverse to elevated recurrence risk, given that PDGF was not significantly associated with the risk of breast cancer recurrence [28]. What was the case was, that only high PDGF levels accompanied by high IGF-I levels, increased the risk of recurrence, suggesting a potential PDGF "priming" effect on IGF-I receptors.

Autologous fat grafting

Fat grafting contributes to the form restoration and to the functional restoration for a wide variety of soft tissue defects, such as those arising after tumor excision for breast cancer. Although a variety of fillers and implants have been used to improve the aesthetic result after a breast surgery, Autologous Fat Grafting (AFG) remains a highly recommended method for small to medium sized breast defects, while avoiding the emerging problems of the other autologous- or heterologous- derived biologic materials, as well as reducing the danger of inflammation and infection of the foreign materials used. Additionally, AFG provides an aesthetic result closer to that of normal soft tissue.

However, a major defect of AFG remains the maintenance of the provided graft volume. Currently, long term results of maintenance vary widely, with an observed retention rate from 30 to 70%; resulting patients undergoing multiple operations and staged procedures [29]. Disadvantages of AFG include fat necrosis, calcifications and oil-cyst formation other than graft volume retention. As a result, improving the fat graft survival and constitution remain the strongest challenges for the researchers.

To this direction, cell-assisted lipotransfer (CAL) was the initial act attempted in the past decade to address the problem of fat graft survival and volume maintenance [30]. Cell assisted lipotransfer (CAL) uses isolated cells coming from the adipose-derived stem cell-rich Stromal Vascular Fraction (SVF), adding the adipose-derived stem cells to the fat graft aspirated. Kolle et al. [31] supported that CAL improves the graft survival both via en-

hanced cell differentiation and secretion of vascular growth factors.

Adipose-derived Stem Cells (ACSs) are mesenchymal stem cells similar to those found in the bone narrow, differentiate into a wide range of cells that can be extracted and cultured from a lipoaspirate [32]. Adipocytes, osteocytes and chondrocytes are all cells originating from the ASCs [33-35]. Furthermore, ASCs release vascular growth factors, which have a major part supporting the grafted tissues.

On the other hand, the clinical application of stem cells remains quite difficult. While the process of ASCs culture takes a great amount of time to be carried out, they cannot be used in the clinical practice at the exact same time. Comparing to cultured and relatively homogenous ASCs, an uncultured heterogeneous SVF is easily available to clinical practice without the long ASCs cultivation period. Both SVF and ASCs can be extracted with lipoaspirates, but unlike SVF contains a stromal component, including lymphocytes, monocytes, granulocyte, endothelial cells, and pericytes [36]. The ASCs are extracted from this SVF population. The addition of SVF to fat grafts is used as an improvement to graft reliability in the field of regenerative medicine [37].

Contradictions of the use of ASCs

These mixed results are similar to the situation with the use of adipose-derived stem cells (ASCs) in AFG and related techniques in breast reconstruction: some preclinical studies [38-41] have found that ACS-associated immunosupression may favor tumor cell proliferation, including of active BC cells and prostate cancer cells. In contrast, several other preclinical studies [42,43] have shown an antitumor / antiproliferative capability from these same ACSs in certain malignancies like pancreatic ductal adenocarcinoma (PDAC) and human colorectal cancer (CRC), and possibly others. A recent Dutch systematic review [44] suggested that such application was not associated with any increased incidence of breast cancer, although more robust data is required to draw clinically relevant conclusions.

Conclusion

Our data are not totally consistent and have in fact some directly contradictory findings to suggest the long-term oncologic safety of PRP-assisted breast reconstruction. Until safe data is published, caution upon the recent publications is prudent, because this method should be viewed as without sufficient evidence and warrants clinical deployment in the current breast oncology context.

Conflict of interests

The authors declare no conflict of interests.

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