Enhancement of autologous fat graft survival by recipient site preparation

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ABSTRACT

Fat grafting has emerged as a very powerful tool, largely used in plastic surgery for a multitude of indications, including correction of contour abnormalities, breast reconstruction, and cosmetic procedures. However, the variability in terms of volumetric stability of the grafted fat and the lack of methods to measure outcomes in a reliable, easy and reproducible manner represent relevant limitations. To improve outcomes, the majority of the investigations has focused on three of the four steps of the fat grafting process, namely harvesting, processing, and re-injection. The preparation of the recipient site has instead received less attention, despite several reports suggesting an ability of determining higher fat survival rates, mainly through induction of cell proliferation, neo-vascularization and neo-adipogenesis. It is therefore of utmost clinical relevance to determine whether the preparation of the recipient site prior to fat grafting leads to more favorable results. The aim of the present work is to collect and review all existing pre-clinical and clinical evidence regarding all methods to prepare the recipient site, to introduce a new preparation technique and to validate a new three-dimensional method to assess outcomes objectively.

Four publications are included. The first manuscript provides a comprehensive overview of the different techniques to prepare the recipient site for fat grafting as they were investigated in pre-clinical studies, including external volume expansion, implantation of alloplastic material, administration of cell-proliferation factors, ischemia, and microneedling. The resulting outcomes are analyzed and the underlying mechanisms of action clarified. The second paper reviews all clinical studies investigating the most used preparation technique, external volume expansion, examining different indications, treatment protocols, outcomes, and complications. The third article presents an innovative and simple intraoperative external expansion system which applies a strong cycling negative pressure of -550 mmHg to enhance small-volume autologous fat grafting (40–80 mL) and discusses its background and its mechanism of action. In particular, this article examines our experience with recipient sites in the breast characterized by restrictive cicatrix or pre-irradiated tissues. Finally, the fourth

study validates the use of a novel, inexpensive and handheld three-dimensional scanning process to perform an easy and precise measurement of breast volumes and surfaces, extremely useful to evaluate fat grafting outcomes, especially in case of small volume transplantation.

I. INTRODUCTION

1.1. *The use of fat grafting in plastic surgery: an overview*

During the past decades, autologous fat grafting (AFG) has become a widely used and well-established procedure in plastic surgery for both reconstructive and aesthetic purposes (Gir et al, 2012; Del Vecchio and Rohrich, 2012; Strong et al, 2015). Indeed, the data recently released by the International Society of Aesthetic Plastic Surgery show that, with more than 1,000,000 procedures performed in 2016 over a total of 10,000,000, AFG is one of the most used for breast and gluteal augmentation and facial rejuvenation (ISAPS, 2016). A key aspect in the success of the procedure is represented by the abundance of adipose tissue in the body which can be removed form a donor site presenting unpleasant and excessive accumulation to a recipient site lacking volume (Oranges et al, 2018). Moreover, AFG is gaining a very relevant role in the field of regenerative medicine due to the proven regenerative potential displayed by the grafted fat. This effect is mainly attributed to the stromal vascular fraction (SVF) and already applied in the clinical setting for the treatment of scars, scar-related conditions, and burns (Oranges et al, 2018; Negenborn et al, 2016; Condé-Green et al, 2016).

However, among its limitations a special place is occupied by a current huge variability in terms of graft survival (30–80%) observed by different authors who used different methods (Gir et al, 2012). In order to guarantee the success of the operation and allow high retention rates and even large-volume transplantation, it is certainly essential to apply techniques that abide by the principles of AFG (Khouri RK Jr and Khouri RK, 2017).

Therefore, the seek for evidence in AFG is motivated by the desire of establishing an ideal surgical strategy, which may guarantee optimal outcomes. To this end, the main focus has been concentrated on three of the four phases of AFG, namely fat harvesting, processing, and reinjection. Both pre-clinical and clinical studies have extensively analyzed these three phases. The evidence contained in these researches has been reviewed by Strong et al in 2015 (Strong et al, 2015).

The fourth phase of recipient site preparation, instead, has mostly been neglected (Gir et al, 2012; Strong et al, 2015; Oranges CM et al, 2018). Indeed, the recipient site has been correlated to the success of fat grafting mainly for its intrinsic characteristics, such as the age of the patient, mobile versus less mobile areas of the face, trauma, burns, scars, structural defects, and compartments on the face (Rohrich et al, 2007; Wang et al, 2017; Oranges et al, 2017).

1.2. *The preparation of the recipient site in fat grafting*

Although much less studied, the recipient site preparation has generated great interest especially with regard to the application of external expansion techniques. This has been correlated in particular to the very positive results reported for the use of the Brava system (Brava LLC, Miami, Fla.), a bra-like external expansion device which prepares the breast before AFG (Khouri and Del Vecchio, 2009). The system was described as extremely promising, with authors such as Del Vecchio and Bucky reporting 60%–200% increase of human breast volume after autologous fat injection documented by quantitative magnetic resonance imaging (Del Vecchio and Bucky, 2011). One of the main indications for the use of Brava is the need for mega-volume (>250 cc) fat grafting in and around the breast (Khouri RK et al, 2012), which in the opinion of the researchers could not be possible without the vacuum preparation of the recipient site.

Due to its already relevant clinical use, several pre-clinical studies have been performed to clarify the mechanism of action of external volume expansion. Heit et al (Heit et al, 2012) and Lancerotto et al (Lancerotto et al, 2013) have demonstrated the induction of significant cell proliferation and neo-angiogenesis in the subcutaneous tissue after exposing mouse integument to external volume expansion, elicited even by a single two-hour external volume expansion cycle.

However, among the limitations to the use of Brava, there is the need of wearing the device for a minimum of 10 hours per day during the weeks before the operation, which requires a considerable compliance and motivation of the patient. Moreover, many conditions only demand limited interventions with small amount of fat grafting to correct contracted scars and body contouring deformities. The beneficial effect of the preparation of the recipient site, in particular the release of the contracture at the time of AFG has indeed generally been considered necessary in cases of contracted scar tissue, as seen in breasts with previous infection, radiation scarring, scar contracture from previous surgery, or congenital constriction bands, as seen in tuberous breasts (Khouri RK et al, 2014). In an attempt to achieve this result, many surgeons currently perform “Rigottomies,” named after Gino Rigotti, who described the technique, which release and stretch-expand scars by creating multiple tiny nicks inside the contracted tissue (Khouri RK et al, 2014). The scar is consequently transformed into a three-dimensional mesh, the volume of the recipient bed is expanded, and the interstitial fluid pressure reduced to the adequate range for graft survival (Khouri RK et al, 2014).

Although external expansion is certainly the most popular, other techniques to prepare the recipient site prior to fat grafting exist and were investigated clinically and pre-clinically. (Wang et al, 2017; Oranges et al, 2017; Zocchi et al, 2008). However, the available evidence was never reviewed. A comprehensive analysis of the literature is of extreme importance to objectively understand the contribution of the preparation of the recipient site and establish the basis to future investigations. Moreover, the need of treating in a simple and inexpensive way limited areas of the breast motivates the search of innovative strategies.

1.3. *Objective evaluation of fat grafting outcomes and use of three-dimensional scanning processes*

One of the major limitations of the research in AFG is the lack of standardized and reproducible methods to assess outcomes in an objective and easy manner. This applies particularly well to the post-operative volumetric changes able to indicate retention rate and stability of the grafted fat. An area where this appears to be of utmost relevance is certainly breast surgery, because of the large use of AFG in this context and the possibility of standardizing the measurement process in this anatomical region.

However, several investigations have validated the use of three-dimensional photographic imaging technologies in the clinical context of autologous or prosthetic breast reconstruction, breast augmentation and breast reduction, with expected future applications also in case of AFG (Losken et al, 2005; Kovacs et al, 2006; Isogai et al, 2006; Tepper et al, 2006; Tepper et al, 2008; Tepper et al, 2009; Choi et al, 2009). These technologies are overall increasingly used in plastic and reconstructive surgery, as they allow efficient pre-operative evaluations, accurate diagnosis based on a deep understanding of the underlying morphology, and appropriate surgical planning based on accurate establishment of endpoint goals of treatment (Pfaff and Steinbacher, 2016).

Another advantage offered by three-dimensional imaging as a powerful tool to objectively measure outcomes in comparison with traditional two-dimensional photography, is its ability of providing important additional data, such as true surface anatomy and volumetric and geometric parameters, including depth and surface topographic distance measurements (Weissler, 2017). These elements are of utmost relevance in breast surgery, where they can be used to evaluate symmetry, surface and volumetric changes, total breast volumes, volumetric distribution, and breast projection. Furthermore, breast contour, size, and position on the chest wall can be defied by surface and vector measurements (Tepper et al, 2010).

However, many of the devices validated for three-dimensional breast images capturing are of unpractical use in daily practice, due to their not portability, heaviness and overall expensiveness (Koban, 2018). Our group has previously described the use of a novel, simple and inexpensive three-dimensional scanning system for breast surface evaluation (Oranges, 2018). Nevertheless, our study did not validate its use in comparison to other clinically established three-dimensional imaging systems.

1.4. *Research objectives*

The present PhD thesis aims at investigating the preparation of the recipient site prior to AFG, its ability to improve outcomes and the complications possibly associated with its use. This appear to be extremely important, as the extreme variability in terms of fat graft survival reported in literature has already largely been explored in relation to the other phases of the procedure, while the preparation of the recipient site is less investigated.

The thesis explores overall the preparation of the recipient site with a specific focus on pre-expansion, presents a new method of external volume expansion and validates an innovative three-dimensional imaging scanning process which may be particularly useful in case of AFG outcomes assessment.

First, a comprehensive review of the pre-clinical evidence on the preparation of the recipient site is performed to clarify the mechanism of action of the techniques already used clinically and to provide an overview of the techniques whose preclinical positive outcomes will lead to future clinical applications. Then, a second manuscripts presents a comprehensive analysis of the international literature regarding all of the studies which investigated recipient site preparation using external expansion. These works are followed by a study on a novel external expansion technique to prepare the recipient site by applying a strong cycling negative pressure of -550 mmHg. The intra- and post-operative use of the procedure in case of recipient sites characterized by contracted scars or body contouring

deformities are reported and discussed with resulting outcomes and complications. Finally, a fourth manuscript validates a novel and simple three-dimensional imaging scanning process which allows volumes and surface distances assessment in the breast using an handled and inexpensive device. The implications for objective AFG outcomes assessment, especially in case of small volume transplantation, are examined and discussed.

II. PUBLICATIONS

2.1. **Manuscript 1**

The Preparation of the Recipient Site in Fat Grafting: A Comprehensive Review of the Preclinical Evidence.

Journal: Plastic and Reconstructive Surgery - published

Authors: Oranges CM, Striebel J, Tremp M, Madduri S, Kalbermatten DF, Harder Y, Schaefer DJ.

Abstract - *Background:* Several methods to prepare the recipient site in fat grafting have been proposed in recent decades. However, to date, these procedures have never been reviewed exhaustively. The purpose of the present study is to provide a comprehensive overview of the different techniques to prepare the recipient site for fat grafting as they were investigated in preclinical studies, with resulting outcomes and underlying mechanisms of action. *Methods:* The PubMed/MEDLINE database was queried to search for preclinical investigations on the preparation of the recipient site in fat grafting using the following algorithm: ((recipient site) AND (fat grafting) OR (lipofilling) OR (lipograft)). A priori criteria were applied to review the resulting articles. *Results:* Thirteen animal studies met inclusion criteria. Overall, five techniques were identified: external volume expansion, implantation of alloplastic material (silicone sheets), administration of cell-proliferation factors (i.e., vascular endothelial growth factor, adipose tissue–derived stromal vascular fraction, and interleukin-8), ischemia, and microneedling. A positive effect on cellular activity (cell proliferation and angiogenesis) was demonstrated by all studies and achieved with all techniques. Seven of the eight authors who examined this aspect reported enhancement of fat graft survival. *Conclusions:* Improvement of fat grafting surgical outcomes is documented preclinically using different recipient-site preparation techniques, particularly through enhancement of vascularization and soft-tissue expansion. This understanding will lead to further clinical research, especially for those cases where improvement of the recipient site is recommended, such as contracted scars or preirradiated tissues.

Authorship statement: CMO and JS have a shared first authorship. CMO designed the study and interpreted the data. CMO and JS collected and analyzed the data, wrote the manuscript and made tables. CMO corrected and reviewed the manuscript. As corresponding author, CMO took care of the submission and revision process of the manuscript.

The Preparation of the Recipient Site in Fat Grafting: A Comprehensive Review of the Preclinical Evidence

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Background: Several methods to prepare the recipient site in fat grafting have been proposed in recent decades. However, to date, these procedures have never been reviewed exhaustively. The purpose of the present study is to provide a comprehensive overview of the different techniques to prepare the recipient site for fat grafting as they were investigated in preclinical studies, with resulting outcomes and underlying mechanisms of action.

Methods: The PubMed/MEDLINE database was queried to search for preclinical investigations on the preparation of the recipient site in fat grafting using the following algorithm: ((recipient site) AND (fat grafting) OR (lipofilling) OR (lipograft)). A priori criteria were applied to review the resulting articles.

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Conclusions: Improvement of fat grafting surgical outcomes is documented preclinically using different recipient-site preparation techniques, particularly through enhancement of vascularization and soft-tissue expansion. This understanding will lead to further clinical research, especially for those cases where improvement of the recipient site is recommended, such as contracted scars or preirradiated tissues. (*Plast. Reconstr. Surg.* 143: 1099, 2019.)

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Autologous fat grafting is commonly used in plastic surgery for both reconstructive and aesthetic purposes.[1–3] Adipose tissue represents indeed a large source of tissue easily transferred from a donor site with excessive unpleasant accumulation to a recipient site in need of volume enhancement.[4] Moreover, the grafted fat is able to express a proven regenerative potential through its stromal vascular fraction, already applied in the clinical setting for the treatment of scars, scar-related conditions, and burns.[4–6] However, it is essential to apply techniques that abide by the principles of fat grafting to ensure high retention rates, which may be consistently achieved even for large-volume transplantation.[7]

Disclosure: *None of the authors has a financial interest in any of the products, devices, or drugs mentioned in this article.*

In an effort to define an optimal surgical strategy, many researchers have focused particularly on investigating three of the four phases of fat grafting (i.e., fat harvesting, processing, and reinjection), whereas less attention has been generally given to recipient-site preparation.[1,3,4] The first three phases were analyzed extensively in preclinical and clinical studies and reviewed by Strong et al. in 2015,[3] and the preparation of the recipient site was recently investigated by our group in a comprehensive review of all clinical applications of external volume expansion.[4]

Overall, our research reviewed 1274 treated patients from 14 clinical studies.[4] Thirteen of these studies used the Brava system (Brava, LLC, Miami, Fla.), a bra-like external volume expansion device particularly useful to allow large-volume fat transplantation (>200 ml in almost the totality of the cases).[4] With the application of a low negative pressure ranging from a minimum of −15 to a maximum of −80 mmHg for 10 to 24 hours/day for up to 4 weeks before surgery, Brava was used for a multitude of indications, such as breast reconstruction, breast augmentation, correction of iatrogenic or congenital deformities, and the wish to replace preexisting implants. In these studies, fat graft survival ranged between 53 and 82 percent.[4] As an alternative external volume expansion procedure, intraoperative use of a much smaller device (Kiwi VAC-6000 with a Palmpump; Clinical Innovations, South Murray, Utah) that applies a much stronger negative pressure (−550 mmHg) has also been suggested, to prepare recipient sites characterized by contracted scar and radiation therapy sequelae.[8] Because of its already relevant clinical use, the mechanism of action of external volume expansion has already been investigated in numerous preclinical studies.

However, other techniques to prepare the recipient site before fat grafting exist and were presented in clinical or preclinical studies.[9–12] The aim of the present work is to review the preclinical evidence on the preparation of the recipient site to clarify the mechanism of action of the techniques already used clinically and to provide an overview of the techniques whose preclinical positive outcomes will lead to future clinical applications.

MATERIALS AND METHODS

A comprehensive review of the entire PubMed/MEDLINE database was performed between May and June of 2017 to collect the preclinical evidence on fat grafting recipient-site preparation techniques. Articles were initially selected by applying the following search algorithm: ((fat grafting) OR (lipofilling) OR (lipograft) AND (recipient site)).

We included all preclinical studies that used a preparation technique of the recipient site before fat grafting or investigated their biological effects. Exclusion criteria were literature reviews and descriptive articles with no measurable endpoint. There were no restrictions on time or language of publication. References of the publications identified initially were screened to add additional studies. Two independent assessors (C.M.O. and J.S.) reviewed manually all articles and extracted data from the publications, and a third assessor (M.T.) examined the articles in case of disagreement. All types of preparation techniques were considered, including external volume expansion, alloplastic material implantation, administration of cell-proliferating factors, ischemia, and microneedling. We documented and tabulated the following information for each article: author name(s), year of publication, preparation technique, animal model, comparator, and outcomes/findings.

RESULTS

The initial search returned 117 full-text articles, 105 of which were excluded after applying the predetermined criteria. One article was included after reviewing references of the publications identified initially. Therefore, 13 animal studies published from 1992 through 2017 were finally included in the analysis (Table 1).[13–25]

Five preparation techniques were identified: (1) external volume expansion, performed in six animal studies (122 mice, three pigs, and 20 rabbits)[13,15–17,19,20]; (2) implantation of alloplastic material (silicone sheets), in one animal study on 25 rabbits[24]; (3) administration of cell-proliferating factors, namely vascular endothelial growth factor (VEGF), adipose tissue–derived stromal vascular fraction, and interleukin (IL)-8, in three animal studies (20 mice and 24 rats)[21–23]; (4) ischemia, in one animal study (mice)[14]; and (5) microneedling, in two animal studies on 38 rats.[18,25]

Outcome evaluation was performed considering effects on fat graft survival, vascularity, cell proliferation, skin thickness, quality of tissue, and inflammation. The preclinical outcomes and mechanism of actions of the five preparation techniques are extensively presented below in separate paragraphs.

External Volume Expansion

With this method, an external expander is applied to the recipient site.[4] Overall, six preclinical

Table 1. Overview of the Studies Investigating the Preparation of the Recipient Site before Fat Grafting

Reference	Technique of Recipient-Site Preparation	Animal Model	Outcomes/Findings	Recipient Site	Comparator
Giatsidis et al., 2017[13]	External expansion; continuous pressure of −25 mmHg; duration ranged from 1.5, 1 time/day for 1 day to 1.5 hours, 5 times/day for 5 days	50 mice	Increase of cutaneous vascular density and induction of skin thickening with expansion of the subcutaneous tissue; complications: tissue damage after high-intensity intermittent stimulations	Mice dorsum	Contralateral, non-stimulated side of the dorsum
Gassman et al., 2016[14]	Ischemia; intermittent temporary hindlimb tourniquet application on donor and recipient sites before harvest and transfer	Mice, number not reported	Approximately 2- to 9-fold increase in bioluminescence; substantially less interstitial fibrosis	Mice subcutaneous dorsal skin folds	Contralateral, non-treated side of the dorsum
Hsiao et al., 2016[15]	External expansion; continuous negative pressure of −70 mmHg; 4 groups: 1 or 3 hr applications every other day for 10 or 20 days	3 swine	Increase in epidermis thickness, vessel density, and cell proliferation	Swine dorsum	Contralateral, non-expanded side of the dorsum
Lujan-Hernandez et al., 2016[16]	External expansion; continuous pressure of −25 mmHg for single 2-hr stimulation or for 2 hours daily for 5 days	28 mice	Increase in the number of adipocytes per square millimeter with increase of PPAR-γ expression; edema in the immediate poststimulation period, and elevated inflammation for 2 days	Dorsum, 1 cm lateral to the spine	Contralateral, non-expanded side of the dorsum
Lee et al., 2015[17]	External expansion; continuous pressure of −125 mmHg	20 rabbits	Increase in skin blood flow rate, viability, and microvessel density; no difference concerning survival rate of the fat graft	Dorsal ear	Contralateral ear
Sezgin et al., 2014[18]	Microneedling; standard microneedling technique with Deeproller 1.50 mm followed by fat grafting	18 rats	Increase in maintenance of volume and vascularity; better structural integrity and lobulation of the adipocytes	Dorsal subcutaneous pouch	Control group and sham group
Lancerotto et al., 2013[19]	External expansion; continuous pressure of −25 mmHg for 2 hr; no fat grafting performed	24 mice	Increase in epidermal and dermal cell proliferation and vascular density; inflammation elevated for 2 days; complications: development of macroscopic swelling, with intense edema	Dorsal skin 5 cm cephalad to the tail and 3 cm lateral to the midline spine	Control group of untreated mice
Heit et al., 2012[20]	External expansion; continuous pressure of −25 mmHg for 28 days	20 mice	Increase in thickness of the subcutaneous fat layer and vascularity, proliferation of adipocytes	Dorsal skin, 3 cm cephalad to the tail and 0.5 cm lateral to the midline spine	Control group of untreated mice
Topcu et al., 2012[21]	Administration of cell proliferating factors; microspheres filled with VEGF before (group 1) or at time of implantation of fat (group 2) or empty spheres at time of implantation (group 3)	24 rats	Increase in weight of transplanted fat, vascularity, and relative adipocyte indices	Dorsal interscapular region	Control group
Koh et al., 2011[22]	Administration of cell proliferating factors; four groups according to whether the coinjected Matrigel contained: 1. Bovine serum albumin 2. VEGF 3. SVF only 4. SVF with vascular growth factor supplements	Mice, number not reported	Increase of adipocyte content was maximal when VEGF and SVF were co-implanted	Flank	None

(*Continued*)

Table 1. *Continued*

Reference	Technique of Recipient-Site Preparation	Animal Model	Outcomes/Findings	Recipient Site	Comparator
Shoshani et al., 2005[23]	Administration of cell proliferating factors; IL-8 (0.25 ng) was injected subcutaneously to the scalp 24 hr before the fat injection and was added to the fat graft itself	20 mice	Less cyst formation; no differences in graft weight, volume, adipose cell sizes, and triglyceride content or the other histologic parameters investigated	Scalp	Control group
Baran et al., 2002[24]	Alloplastic materials; insertion of silicone sheets in recipient areas, excision of the sheets and parts of the formed capsule after 3 wk; transplantation of two types of fat tissue: in one, lobular structure was preserved, in the other one, fat was manually crushed and rinsed with lactated Ringer solution	25 rabbits	Slower absorption rate in treated areas; increase in volume maintenance	Dorsum	None
Samdal et al., 1992[25]	Microneedling; 20 strokes of an 18-gauge needle in a criss-cross pattern	20 rats	Increase in transplanted blood flow and weight of transplant	Skin over right pectoral muscle	Control group

PPAR-γ, peroxisome proliferator-activated receptor-γ; VEGF, vascular endothelial growth factor; SVF, stromal vascular fraction; IL-8, interleukin-8.

studies were conducted with a wide variability in terms of negative pressure applied.[4,26] A negative pressure of −25 mmHg was used in mice models by four authors, three of which observed stretch, edema, and inflammation of tissues, elements responsible for cell proliferation and neoangiogenesis.[4,13,16,19,20] Consequently, a proadipogenic effect with higher density of subdermal adipocytes was reported. In detail, an increase in inflammatory $CD45^+$ cell density ($p < 0.01$) or onset of inflammation already apparent by the end of stimulation were observed by Lancerotto et al. and Lujan-Hernandez et al.,[16,19] whereas Giatsidis et al. reported no difference ($p > 0.05$).[13] Improvement in cell proliferation was reported by all four studies, which observed an increase of the following factors: (1) number of adipocytes, with 1.5- to 2.2-fold increase over control following external volume expansion ($p < 0.05$)[13,16,20]; (2) peroxisome proliferator-activated receptor-γ expression, equal to 1.0 ± 0.17 after external volume expansion versus 0.24 ± 0.05 in the control group ($p < 0.01$)[16]; (3) proliferating rate of cells located in the dermis and epidermis, with 61 to 85 percent of Ki67-positive cells in the study group versus 36.4 percent in the control group ($p < 0.01$)[15]; and 1.4- to 1.9-fold higher proliferation rate at cell count after external volume expansion ($p < 0.05$).[19,20] A significant increase of skin and subcutaneous tissue thickness was observed by two authors and reported as follows: 1.9-fold increase of skin thickness ($p < 0.01$),[13] and 2-fold increase of the subcutaneous thickness ($p < 0.05$).[20] Increased vascularization was documented by Heit et al. and Giatsidis et al. and equaled, respectively, 1.5- and 1.9-fold increase ($p = 0.01$) of cutaneous or subcutaneous tissue blood vessel density,[13,20] whereas Lancerotto et al. reported 49 vessels/high-magnification field in the study group versus 35 vessels/high-magnification field in the control group ($p < 0.01$).[19]

A lower pressure equal to −70 mmHg was applied by Hsiao et al. to the dorsum of pigs for 1 hour/day for 10 days, 1 hour/day for 20 days, 3 hours/day for 10 days, or 3 hours/day for 20 days, respectively.[4,15] The study demonstrated increased cell proliferation, vascularity, skin thickness, and skin loosening. The effects were stronger in the groups with longest exposure. Specifically, a higher proliferating rate of cells located in the dermis and epidermis was observed, with 61 to 85 percent of Ki67-positive cells after external volume expansion versus 36.4 percent in the control group ($p < 0.01$). The increased vascularization, expressed as increased blood vessel density, was

documented with 6.2 to 9.4 blood vessels/mm^2 in the treated area versus 4.2 blood vessels/mm^2 in the control area ($p < 0.001$). Skin thickness increase, with increase of the subcutaneous thickness, was documented by 63- to 90-μm epidermal tissue thickness in the treated area versus 49 μm in the control area ($p < 0.05$). The severity of skin loosening seemed to increase with longer application of the external expansion device.

Finally, Lee et al. chose to test a much lower pressure (−125 mmHg), equal to the negative pressure used for noninvasive wound closure.[4,17,27] Treating the dorsal ear of 20 white rabbits for 1 week before fat grafting, they obtained recipient-site enhanced vascularization and increased fat graft survival.[4,17,27] Specifically, they reported a statistically significant increase of graft survival in the study group versus the control group (75.4 ± 3.9 percent versus 53.1 ± 4.3 percent, respectively; $p < 0.001$) and a statistically significant increase of glycerol expression, used to determine adipocyte viability (183.56 ± 55.1 μg/ml/400 mg in the study group versus 100.32 ± 32.18 μg/ml/400 mg in the control group; $p = 0.002$).[17] In this study, tissue perfusion was evaluated using laser Doppler flowmetry and was equal to 19.98 ± 3.92 perfusion units in the study area versus 12.71 ± 3.23 perfusion units in the control area ($p = 0.02$).

Another relevant aspect examined was the proper selection between cyclical and static pressures, as cyclical use of negative pressure has been previously demonstrated to provide a more robust response.[17,27,28] Although in the clinical setting either cycling or continuous pressures were applied, all preclinical studies investigated the effects achieved using continuous pressure.[4]

The last aspects considered were the effects resulting from different time and durations of preexpansion.[27] In several studies, animals were divided into groups with different exposure time ranging from one single session of 1.5 hours to 24 hours for 28 consecutive days.[4,15,17,19,20] External volume expansion was only applied before fat grafting in animal studies. No difference statistically significant was found between tissues treated with single 2-hour stimulation or 2 hours per day for 5 days.[16]

Administration of Cell Proliferating Factors

Three investigations conducted on mice and rats assessed the effects of the injection of different cell proliferating factors to the recipient site.[21–23] Topcu et al. injected microspheres filled with VEGF before (group 1) or at the time of fat grafting (group 2) into the dorsal interscapular region of rats.[21] They observed significantly better outcomes in terms of weight of the transplanted graft and vascularity in the study groups compared with control. In detail, the study showed a statistically significant increase of graft weight that equalled 141.6 percent when VEGF was added at the time of fat transplantation and 125.3 percent when VEGF was added before fat transplantation, versus 44 percent in the control group ($p < 0.05$).[21] Regarding blood vessel density, they observed 7.92 to 24.42 vessel counts per microscope area after administrations of VEGF microspheres versus 6.59 in the control group ($p < 0.001$).[21]

Koh et al. co-injected matrigel containing bovine serum albumin, VEGF, and stromal vascular fraction into the recipient site.[22] The authors postulated that enriching the vascular supply with co-implantation of stromal vascular fraction might improve the microenvironment and survival of the graft. Regarding cell proliferation, adipocyte content increased 17.8-fold when VEGF was added, 33.6-fold with stromal vascular fraction, and 40.8-fold with both factors combined ($p < 0.05$).

Finally, Shoshani et al. assessed the effect of IL-8 on the viability of injected adipose tissue in a murine model.[23] The study showed no significant improvement in terms of fat graft weight (0.34 ± 0.20 g in the study group versus 0.44 ± 0.16 g in the control group; $p = 0.23$) and volumes (0.44 ± 0.23 ml versus 0.43 ± 0.25 ml in controls; $p = 0.90$). Also, cell proliferation, evaluated as induction of adipogenesis and measured as triglyceride concentration, did not improve significantly ($p = 0.916$).[23] However, the authors demonstrated significantly less cyst formation in the group treated with IL-8 ($p = 0.037$).[23]

Ischemia

Gassman et al. investigated the viability of the grafted fat in remote ischemic preconditioned recipient sites of mice.[14] Donors and recipients experienced intermittent temporary hindlimb tourniquet application (three cycles of 5 minutes' ischemia) before harvest and transfer of fat, respectively. The authors were able to show an increase in viability and substantially less interstitial fibrosis and liponecrosis in the preconditioned study group ($p < 0.05$). Fat graft viability was measured with bioluminescence and was retained at 300 percent after ischemia versus 30 percent in the control group ($p < 0.05$).[14] This was correlated to improved distant capillary bed tissue oxygen saturation and perfusion, which limited ischemic injury to the transferred tissue.

Microneedling

Two preclinical studies were performed using microneedling in rat models with the following protocols: Sezgin et al. performed microneedling with a device called Deeproller 1.50 mm (Assos Pharmaceuticals, Istanbul, Turkey) equipped with 192 microneedles of 1.5-mm length, applying 20 back-and-forth rolling movements with standard pressure execution both parallel and perpendicular to the axis of the rat's dorsum.[18] Samdal et al. abraded the subcutaneous tissue over the right pectoral muscle with 20 strokes of an 18-gauge needle in a criss-cross pattern.[25]

The study by Sezgin et al. showed a higher level of vascularity in terms of blood vessel density, documented with 3.35 ± 2.3 capillaries/high-power field following microneedling versus 1.4 ± 1.19 capillaries/high-power field in the control group ($p < 0.01$).[18] The authors also noted significantly less inflammation ($p < 0.05$), cyst-vacuole formation, and fibrosis ($p < 0.01$) in the study group in comparison with the control group.[18] However, the enhancement in adipocyte integrity and lobulation, with improvement of cell proliferation expressed in terms of triglyceride concentration (438.5 ± 148.6 mg/dl in the study group versus 351.2 ± 137.1 mg/dl in the control group), was found to be not statistically significant ($p > 0.05$).[18]

Samdal et al. reported reduced loss of transplanted fat postoperatively (fat survival index of 17 percent in the study group versus 10 percent in the control group; $p < 0.05$) and, regarding tissue perfusion, a transplanted blood flow equal to 0.187 ml/minute/g in the study group versus 0.120 ml/minute/g in the control group ($p < 0.05$).[25]

Alloplastic Materials

Baran et al. inserted silicone sheets in the dorsum of rabbits to induce capsule formation and thus create a recipient site with enhanced vascularity.[24] After 3 weeks, the sheets were removed and the fibrous capsule formed around the silicone sheet partially excised. Subsequently, two different types of fat tissue were transplanted: one with the lobular structure preserved, and the other with fat manually crushed and rinsed with lactated Ringer solution. In the area prepared with silicone sheets and injected with fat with preserved structure, 30 percent of fat tissue was recovered after 1 year. No evidence of fat tissue after 1 year was observed in the control areas.

DISCUSSION

The recipient site plays a key role in the success of a fat grafting procedure.[29–35] However, of the techniques already used clinically to prepare the recipient site, only the mechanism of action of external volume expansion was investigated preclinically, whereas three clinical techniques—tunnelization, internal expansion, and percutaneous fasciotomies—were not.[8–11] Instead, to the best of our knowledge, all other techniques identified by this review were studied only in the preclinical setting, and constitute the background for future clinical applications.

Overall, the studies on external volume expansion allow the most solid conclusions to be obtained. In particular, controlled noninvasive suction demonstrated the ability to determine cell strain, ischemia, and edema, which trigger inflammation and lead to cell proliferation, angiogenesis, and adipogenesis.[13,15–17,19,20] Indeed, this procedure is the only one that has already been extensively applied clinically, showing positive outcomes for breast reconstruction and augmentation.[36,37] Del Vecchio and Bucky[38] summarized the five main reasons in favor of the clinical use of external volume expansion before fat grafting, as follows: (1) increase of overall parenchymal space; (2) reduced interstitial pressure for a given volume of graft injected; (3) breast shape modification through augmentation of contour irregularities before grafting; (4) possible omission of variables such as high-speed centrifugation with resulting shorter operating room times; and (5) angiogenesis, resulting from micromechanical forces on the recipient site. However, there are still limitations to the widespread adoption of this technique.[4] These include primarily the absence of randomized controlled trials comparing autologous fat grafting with and without external volume expansion.[4] Moreover, the need for wearing the device for 10 to 24 hours/day for 2 to 4 weeks preoperatively, according to recent published protocols, implies adequate patient compliance and motivation, and may be responsible for patient social life restriction and dropout rates.[4]

Promising results were shown also with the administration of cell proliferating factors, in terms of improvement of fat graft survival, vascularity (blood vessel density), cell proliferation, and reduction of cyst formation. Topcu et al. administered VEGF, a glycoprotein able of inducing proliferation, migration, and differentiation in endothelial cells, which were proven to have promotive effects on preadipocytes in a paracrine manner by means of either VEGF or some other soluble factors.[21,39–42] Instead, the rationale for using IL-8 was based on its ability to accelerate angiogenesis and attract inflammatory cells and

fibroblasts, providing the injected adipocytes with more feeding vessels and a well-established graft bed to enhance their viability.[43–46] Finally, Koh et al. showed that direct implantation of freshly isolated stromal vascular fraction derived from adipose tissue can induce a rapid and robust process of vascular network formation through the dynamic reassembly of blood endothelial cells at the site of implantation in syngeneic mice.[22]

Gassman et al. studied the effects of remote ischemic preconditioning before fat grafting.[14] This technique has been shown to have protective effects against ischemia for several organ systems.[47–49] Although the exact mechanism behind this technique remains unclear, several studies have hypothesized a complex multimodal mechanism.[14] A previous investigation by Gassman et al. demonstrated that adipose tissue undergoing remote ischemic preconditioning before transfer displays several hallmarks consistent with ischemic tolerance, such as increased viability and reduced liposclerosis,[50] whereas Kraemer et al. showed that remote ischemic preconditioning improves distant capillary bed tissue oxygen saturation and perfusion.[51] Consequently, Gassman et al. suggested that, in essence, this technique prepared the recipient site for tissue engraftment by limiting the ischemic injury to the transferred tissue.

According to Sezgin et al., the mechanism of action of microneedling is to induce a wound-healing process through mechanical microtraumatization, resulting in beneficial effects for fat transplantation.[18,52] The technique consists of creating a multitude of microchannels that penetrate through the dermis, leading to the activation of the inflammatory cascade, similar to the normal wound healing process. Within this triggered complex cascade, fibroblast growth factor, VEGF, platelet-derived growth factor, and transforming growth factor alpha and beta are endogenously induced, resulting in the stimulation of fibroblasts and collagen production within the skin.[53] Samdal et al. instead hypothesized that preoperative abrasion would increase vascularization on the recipient site and thereby increase the chance of graft revascularization.[25,54]

Finally, with alloplastic material implantation, the silicone sheets cause a foreign body reaction, and the resulting chronic inflammatory response delays and reduces the resorption of fat grafts. The outcome is explained with the longer time required by secondary wound healing compared with primary wound healing.[24,55] By applying this method, Baran et al. were able to show that fat transplantation with lobular structure preserved to the well-vascularized areas created with alloplastic material implantation appeared to be a very valid option for contour deformities when compared with artificial implants, even when the fat grafting needed to be repeated once per year.[24] Overall, although clinical translatability appears possible for the procedures not yet applied in humans, further research on the mechanisms of actions and expected outcomes is required for the development of more standardized treatment protocols and guidelines.

CONCLUSIONS

The preclinical studies identified by this review and related to five techniques to prepare the recipient site in fat grafting were able to clarify the mechanism of action of procedures already used clinically or to offer the background for future applications. Positive outcomes in terms of fat graft survival, vascularity, cell proliferation, skin thickness, quality of tissue, and inflammation were differently shown with these techniques. However, among the different methods, only external volume expansion has been extensively applied clinically, whereas other interventions require validation through future preclinical and clinical investigations.

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2.2. **Manuscript 2**

The Impact of Recipient Site External Expansion in Fat Grafting Surgical Outcomes.

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Authors: Oranges CM, Striebel J, Tremp M, Madduri S, Kalbermatten DF, Schaefer DJ.

Abstract - *Background:* The fat grafting process includes the 4 phases of tissue harvesting, processing, recipient-site preparation, and reinjection. Among them, the preparation of the recipient site has never been exhaustively reviewed. We aim to provide a comprehensive overview of the methods to prepare the recipient site through external expansion with the resulting outcomes. *Methods:* PubMed/Medline database was searched for studies on fat grafting recipient site preparation by applying the following algorithm: ((fat grafting) OR (lipofilling) OR (lipograft) AND (recipient site)). A priori criteria were used to review the resulting articles and identify those dealing with external expansion. *Results:* Fourteen studies published from 2008 through 2016 met inclusion criteria (4 case reports, 6 retrospective, and 4 prospective studies), representing 1,274 treated patients. Two devices for preexpansion were used with different protocols: BRAVA system and Kiwi VAC-6000M with a PalmPump. The 13 studies that applied the BRAVA system reported large fat volume transplantation to the breast (average > 200 cc). The most common complications were localized edema (14.2%), temporary bruising, and superficial skin blisters (11.3%), while the most serious was pneumothorax (0.5%). The majority of the studies reported enhancement of fat graft survival, which ranged between 53% and 82% at 6 months to 1 year follow-up, and high satisfaction of patients and surgeon. *Conclusions:* External expansion and fat grafting is a promising technique for breast reconstruction and augmentation. However, due to the overall low level of evidence of the available studies, further research is needed to validate the procedure.

Authorship statement: CMO and JS have a shared first authorship. CMO designed the study and interpreted the data. CMO and JS collected and analyzed the data, wrote the manuscript and made tables. CMO corrected and improved the manuscript. As corresponding author, CMO took care of the submission and revision process of the manuscript.

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SPECIAL TOPIC
Breast

The Impact of Recipient Site External Expansion in Fat Grafting Surgical Outcomes

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Background: The fat grafting process includes the 4 phases of tissue harvesting, processing, recipient-site preparation, and reinjection. Among them, the preparation of the recipient site has never been exhaustively reviewed. We aim to provide a comprehensive overview of the methods to prepare the recipient site through external expansion with the resulting outcomes.
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Conclusions: External expansion and fat grafting is a promising technique for breast reconstruction and augmentation. However, due to the overall low level of evidence of the available studies, further research is needed to validate the procedure. *(Plast Reconstr Surg Glob Open 2018;6:e1649; doi: 10.1097/GOX.0000000000001649; Published online 8 February 2018.)*

INTRODUCTION

During the past decades, autologous fat grafting (AFG) has become a well-established procedure in Plastic Surgery, widely used for both reconstructive and aesthetic purposes.[1–3] According to data released by the International Society of Aesthetic Plastic Surgery, it is indeed 1 of the most common operations for breast and buttock augmentation and facial rejuvenation, accounting for more than 1,000,000 procedures performed in 2016 over a total of 10,000,000.[4] AFG is appreciated for providing an abundant and easily available source of tissue removed from a donor site with excessive unpleasant accumulation to a recipient site in need for volume enhancement. In addition, the proven regenerative potential expressed by its stromal vascular fraction, has been applied for the treatment of scars, scar-related conditions and burns.[5,6]

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Dr. Oranges and J. Striebel contributed equally to this work.

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Notably, recent research has especially focused on 3 of the 4 phases of the procedure, namely fat harvesting, processing, and reinjection, while the additional step of recipient-site preparation has mainly been neglected.[1,3] In particular, harvesting, processing, and reinjection were extensively examined in a recent comprehensive review by Strong et al.[3] published in 2015, which is the most up-to-date available information on AFG.

Conversely, although many considerations were dedicated to the recipient-site preparation and great interest in this regard has been generated by the external expansion techniques, including the use of BRAVA system (Brava LLC, Miami, Fla.),[7] this was never comprehensively or systematically reviewed. However, inter alia, several variables related to the recipient site in itself were already identified and correlated to AFG success (age of the patient, mobile versus less mobile areas of the face, trauma, burns, scars, structural defects, compartments on the face).[8–10]

The seek for evidence in fat grafting is motivated by the desire of establishing an ideal approach, which may guarantee optimal outcomes by understanding the reasons underling the current huge variability in terms of graft survival (30–80%) observed by different authors who used different methods.[1] The aim of the present research is to present a comprehensive analysis of the international literature regarding all of the studies, which investigated recipient-site preparation with a focus on external expansion.

MATERIALS AND METHODS

Between May and June 2017, a literature review of the entire PubMed/Medline database was conducted to assess the efficacy and complications of AFG recipient-site preparation with external expansion. The search algorithm was: ((fat grafting) OR (lipofilling) OR (lipograft) AND (recipient site)).

Inclusion criteria were (1) clinical studies (case reports, retrospective or prospective case series, clinical trials); (2) application of a recipient-site external expansion technique before fat grafting. Excluded from the analysis were literature reviews and descriptive articles with no measurable endpoint.

No restrictions on time or language of publication were applied. References of the publications identified initially were screened to add studies fulfilling inclusion criteria.

All articles were screened manually. Two investigators (C.M.O. and J.S.) independently reviewed and extracted data from the publications, which were examined by a third reviewer (M.T.) in case of disagreement.

All types of external expansion techniques were considered. We documented and tabulated the following information for each article: author name(s), year of publication, external expansion procedure, study design, number of patients, indication for treatment, comparator, and outcomes/findings.

RESULTS

One hundred seventeen full-text articles were initially identified, 110 of which were excluded according to predetermined criteria. Seven articles were included after reviewing references of the publications identified initially. Therefore, our analysis comprised 14 studies, which were published from 2008 through 2016. Fourteen clinical studies on external expansion were performed on 1,274 patients (4 case reports, 6 retrospective, and 4 prospective studies). The maximum level of evidence was found to be equal to 3 in prospective case series. Surgical indications for fat grafting were breast reconstruction after treatment for cancer, breast augmentation for aesthetic purposes, correction of iatrogenic deformities (deformity after excision of a congenital nevus as child and deformity due to a surgical cardiac procedure), correction of congenital deformities, and the wish to replace preexisting implants. A detailed analysis of all studies is reported in Table 1.

Thirteen authors used the Brava system (Brava LLC, Miami, Fla.), a bra-like device generating a low negative pressure (maximum, -80 mm Hg) worn for 10–24 h/d for 2–4 weeks preoperativly.[2,7,11–21] Large amount of fat transplant was allowed, with 7 of the authors who performed megavolume fat transplant (≥ 300 cc)[2,7,11,13–15,17] and almost the totality of cases (n = 1,272) receiving more than 200 cc of fat graft per session in average. One to 7 sessions were performed to achieve satisfactory results. The studies reported a mean fat graft survival ranging between 53% and 82%.[11–14] Three studies, 2 of which compared their outcomes with previous published series, reported significant enhancement of fat graft survival in comparison with fat grafting without preexpansion.[11,12,14]

Brava system was used with a wide range of pressure values. Although some of the studies did not explicitly report on the pressure applied, we hypothesize that they used the device as initially described by Khouri et al.[12,18–20,22,23] (-15 to -25 mm Hg). Kosowski et al.[21] used a pressure cycling between -60 and 0 mm Hg preoperatively and -20 mm Hg postoperatively, whereas Khouri et al.[14] in 2014 applied a pressure cycling between -80 and -60 mm Hg and "low pressure" postoperatively. Finally, Del Vecchio and Bucky[17] stated that expansion programs were individualized for each patient based on lifestyle analysis and psychological compliance testing, with a negative pressure, which in 1 of his studies was ranging from -1 to -3 inches of mercury (-25.4 to -76.2 mm Hg).[15]

Four studies reported the use of cyclical pressure,[14,18,21,24] whereas the remaining studies did not report on whether the device was used with continuous or cycling power, yet we believe that it was continuous as initially described.[22]

Regarding different time and durations of preexpansion, patients started their treatment with the external expansion device up to 4 weeks before autologous fat transfer for 10–24 h/d.[2,7,11,12,14–19] Postoperatively, the Brava system was worn for 5 days to 4 weeks, with the duration of application ranging from 10 to 24 h/d, only at night or for as many hours per day as tolerated.

The most common complications using Brava system were localized edema (14.2%),[11] temporary bruising and superficial skin blisters (11.3%),[11,20] and fat necrosis (8.2%).[11,12,14] The most serious complication was pneumothorax, which occurred in 6 cases (0.5%), 1 of which

Table 1. Overview of the Studies Investigating Recipient Site External Expansion before Fat Grafting

Author	Technique of Recipient Site Preconditioning	Study Design	No. Patients	Outcomes/Findings	Recipient Site	Comparator	Indication
Oranges et al.[24]	Kiwi VAC-6000M with a PalmPump. Cycling pressure of -550 to 0 mm Hg for 10 times for 30 s each intraoperatively; 3 times per day for 3 days postoperatively at a pressure of -550 mm Hg for 1 minute.	Retrospective study	Not reported	Minimal morbidity and high patient acceptance and compliance. Complications: small degree of edema, which resolved without sequelae.	Breast	None	Breast reconstruction (cancer therapy)
Kosowski et al.[21]	Brava system with a cycling pressure of -60 to 0 mm Hg for 10 h/d for 2–3 weeks preoperatively, and with a pressure of -20 mm Hg for 3–4 weeks postoperatively. It was performed on 3 different patient populations: 1. Delayed breast reconstruction: use of Brava 2–3 weeks before surgery. 2. Immediate breast reconstruction: removal of the investing fascia, use of Brava after the first AFT. 3. Reconstruction for lumpectomy defects.	Retrospective study	427 Patients	Patient satisfaction with the volume, contour, and feel of their breasts was equal to 97%. Fat transplantation volume averaged 225 mL per breast per session. Complications were 5 pneumothoraxes, 5 uncomplicated bacterial cellulitis infections, 2 atypical mycobacterial infections requiring multiple debridements, and 18 cases of ulceration necrosis or mastectomy flap necrosis	Breast	None	Breast reconstruction (cancer therapy n = 427)
Hammer-Hansen et al.[20]	Brava system for 10 h/d 4 weeks preoperatively, for 22 h/d for the last week, resumed 8 weeks after AFT.	Case report	1 Patient	Both patient and surgeon evaluated the reconstruction of the breast as optimal with a soft and natural appearing breast. Fat transplantation volumes ranged between 30 cc and 200 cc within 7 sessions. Complications: irritative rash of the skin and evolvement of blisters, subsiding only after discontinuation of Brava.	Breast	None	Breast reconstruction (cancer therapy n = 1)
Khouri et al.[14]	Brava system with a cycling pressure from -80 to -60 mm Hg for 3 min and no pressure for 1 min for 10 h/d for 4 wk preoperatively, 24 h/d for the last 1–2 d; postoperatively "low pressure" for 3–4 wk.	Prospective study	476 Patients	Volume increase through Brava was between 2.2 fold to 2.7 fold the original volume in different patient populations. Fat transplantation volumes averaged 346 mL per breast. Mean volume maintenance was 76.9% at ≥ 6 mo follow-up. 96% of patients were satisfied with their results. Complications: pneumothorax (n = 1), requiring a temporary chest tube; fat necrosis (n = 90), minor infections treated with antibiotics (n = 7).	Breast	AFG in patients undergoing implant-to-fat conversion without Brava (n = 88)	Aesthetic (n = 294), breast reconstruction (congenital deformity n = 45, iatrogenic deformity n = 43), replacement of preexisting implants (n = 6)
Del Vecchio and Del Vecchio[13]	Brava system.	Retrospective study	30 Patients	Volume increase through Brava averaged 200%. Fat transplantation volumes ranged between 400 and 800 cc. Mean volume maintenance was 53% at 12 months follow-up.	Breast	None	Breast reconstruction (cancer therapy n = 6), aesthetic (n = 24)

(*Continued*)

Table 1. Continued

Author	Technique of Recipient Site Preconditioning	Study Design	No. Patients	Outcomes/Findings	Recipient Site	Comparator	Indication
Uda et al.[19]	Brava system with a pressure of -25 to -15 mm Hg for 10 h/d for 4 weeks preoperatively. Daily steroid ointment was applied after each use. Resuming of Brava therapy for 2 weeks postoperatively.	Retrospective study	14 Patients	Mean grafted fat was 256 cc/session/breast. Improvement was higher in the total mastectomy cases than in the breast-conserving surgery cases at ≥ 6 mo follow-up. Complications: fat lysis and cellulitis, treated with a minor incision and drainage and/or oral antibiotics (n = 2), oil cysts (n = 5).	Breast	None	Breast reconstruction (cancer therapy n = 14, after total mastectomy or breast-conserving surgery)
Ho Quoc and Delay[18]	Brava system for 10 h/d for 4 weeks preoperatively, 24 h/d for the last 4 days, and for 10 h/d for 3 weeks postoperatively.	Prospective study	21 Patients	Fat transplantation averaged 256 cc per session. Complications: erythema in 18 patients, pruritus in 14 patients, sleeping troubles in 2 patients, and phlyctena needing a temporary interruption of Brava in 1 patient (for 72 h).	Breast	None	Breast reconstruction (cancer therapy n = 17, iatrogenic deformity due to a surgical cardiac procedure, n = 1), aesthetic (n = 3)
Del Vecchio[15]	Brava system with a negative pressure of -1 to -3 inches of mercury (-25.4 to -76.2 mm Hg) for 2 weeks preoperatively. AFT while simultaneously removing the preexisting prosthesis from the submuscular space.	Retrospective study	12 Patients	Volume increase through Brava was 2.5 fold the original volume (case report of 1 patient). In 1 patient, over 600 cc was transplanted per breast. Breast volume at 9 mo to 1 yr postoperatively was equal to or greater than before operation.	Breast	None	Replacement of preexisting implants (n = 12)
Del Vecchio and Rohrich[2]	Brava system. Two patients used daily expansion for 3 wk preoperatively, 1 of which required 2 additional fat grafting sessions.	Case report	2 Patients	In 1 case, increase in volume was 300 cc, doubling the original breast volume, done in 1 session. In 1 patient, 550 cc of fat were transplanted, in another patient the volume of transplanted fat ranged from 180–390 cc. In another case, 3 sessions necessary for patient satisfaction. Nonirradiated right breast expanded and augmented consistently larger than the left.	Breast	None	Breast reconstruction (cancer therapy n = 1), aesthetic (n = 1)
Khouri et al.[12]	Brava system. 10 h/d for 4 wk preoperatively, 24 h/d for the first 2–3 d postoperatively, afterward for 4 more days only at night.	Prospective study	81 Patients	Mean augmentation volume of compliant patients was higher than in previous published series without preexpansion. Fat transplantation volume averaged 282 mL per breast. Graft survival was 82% at ≥ 6 mo follow-up compared with 55% without Brava. Complications: fat necrosis (13 patients), temporary bruising, and superficial skin blisters that healed uneventfully	Breast	Previous published series	Aesthetic (n = 81)

(*Continued*)

Table 1. Continued

Author	Technique of Recipient Site Preconditioning	Study Design	No. Patients	Outcomes/Findings	Recipient Site	Comparator	Indication
Del Vecchio and Bucky[17]	Brava system. Brava system for 3 wk with individualized expansion programs; 1–2 d after AFT, patients wore their external expansion devices for 2–4 wk.	Prospective study	25 Patients	Average increase in volume of 272 cc at 6 mo follow-up. This represented a 106% increase. Fat transplantation volumes ranged from 220 to 550 cc of fat per session.	Breast	None	Breast reconstruction (micromastia, tuberous breast, Poland syndrome congenital deformity, postexplantation deformity)
Del Vecchio[16]	Brava system for ≥ 12 h/d for 3 wk, 24 h/d for the last 3 d preoperatively, postopertively for 10 d.	Case report	1 Patient	Volume increase through Brava was estimated as 300%, persistent at 6 mo. 220 mL of fat were transplanted in 1 session.	Breast	None	Breast reconstruction (iatrogenic deformity after excision of a congenital nevus as child, n = 1)
Khouri and Del Vecchio[7]	Brava system for 12 h/d for 3–4 wk, 24 h/d for the last 4–5 d preoperatively, postoperatively for 5–7 d.	Case report	3 Patients	Adequate volume maintenance at 6/9 mo follow-up. In 1 patient, 150 mL of fat were transplanted per session (4 sessions overall), in 1 patient 300 mL were grafted to 1 breast, and in 1 patient 250 mL were grafted per breast.	Breast	None	Breast reconstruction (cancer therapy n = 1, iatrogenic deformity after excision of a congenital nevus as child, n = 1), aesthetic (n = 1)
Zocchi and Zuliani[11]	Brava system for 12 h/d for 30 d.	Retrospective study	181 Patients	Grafted fat volume averaged 375 mL/breast, and average volume persistence at 1 yr was 55%. Complications: localized edema (n = 181), slight bruising (n = 143), dysaesthesia (n = 14), microcalcifications (n = 7), pseudocysts that resolved spontaneously (n = 3), liponecrosis (n = 2).	Breast	None	Aesthetic

required chest tube.[14,21] A complete list of complications observed with Brava system and AFG is reported in Table 2.

In 1 article from our group, it was used a smaller device called Kiwi VAC-6000M with a PalmPump (Clinical Innovations, South Murray, Utah), a complete vacuum delivery system, which applies a stronger cycling negative pressure (-550 mm Hg) for a much shorter intraoperative period (10 times for 30 seconds each) on localized scarred recipient sites before autologous fat injection.[24] Postoperatively, the Kiwi VAC was applied 3 times per day for 1 minute each for 3 days. The authors reported a gross expansion of tissue, with a macroscopic swelling that regressed slowly after the end of the stimulation, and a small degree of edema, which resolved without sequelae as complication. They also observed satisfactory clinical outcomes, with minimal morbidity and high patient acceptance and compliance.

DISCUSSION

The preparation of the recipient site was recently reported as extremely relevant and commonly performed by surgeons treating contracted scar tissues.[25,26] This aspect was extensively examined by Khouri et al.[25] in a publication appeared in 2014 in this journal, which presented a comprehensive overview of fat grafting practice and techniques.[26] Accordingly, the preparation of the recipient site was endorsed for the treatment of either contracted scar tissue or congenital constriction bands to allow a "cicatrix-to-matrix" transformation, with a release of contracture at the time of fat grafting.[25,26]

However, our review is the first that comprehensively reviewed the studies investigating the outcomes achieved with preexpansion performed for different indications and with different protocols. With our inclusion criteria, we identified 14 clinical articles describing the use of 2 external expansion devices, namely Brava system and Kiwi VAC-6000M with a PalmPump, 13 of which reported the treatment of 1,274 patients with Brava system.

The mechanism of action of preexpansion was investigated by 7 preclinical studies, which analyzed the impact of 4 variables: the value of the negative pressure applied, the strength of cycling versus static pressures, the duration and the timing of the preexpansion.

Four authors used a negative pressure of -25 mm Hg, equal to the most commonly used with Brava, in a mice model, observing tissue stretch, edema, and inflammation, factors which triggered cell proliferation, neoangiogenesis, and neoadipogenesis.[24,27–30] Similarly, with a higher pressure (-70 mm Hg) applied to the dorsum of pigs, Hsiao et al.[31] showed an increase in vascularity, cell proliferation, hair follicles number, and skin thickness, yet observing simultaneous skin loosening. Finally, Lee et al.[32] applied a pressure of -125 mm Hg to the dorsal ear of 20 white rabbits for 1 week before fat grafting with the rationale of using the same negative pressure applied for noninvasive wound closure.[33] They reported increased vascularization of the preexpanded recipient site and, accordingly, enhanced fat graft survival.[32,33]

Regarding the comparison between cyclical and static pressures, Chin et al.[34] demonstrated that cyclical use of negative pressure provides a more robust response in terms of epidermal proliferation and angiogenesis.[32]

Finally, in relation to different duration and timing of exposure, in animal studies this ranged from 1 single application of 1.5 hours to 24 hours for 28 consecutive days.[27–33] Notably, the animal study by Lujan-Hernandez et al.[30] showed no significant difference in terms of neoadipogenesis between tissues exposed to single 2 hours stimulation or to 2 hours daily for 5 days.[33]

Brava was initially described for nonsurgical breast augmentation by Khouri et al.[22] in 2000 to exploit the ability of tissues to grow when subjected to controlled distractive mechanical forces. The patients of this initial study were asked to wear a brassiere-like system with 20 mm Hg vacuum distraction force to each breast for 10–12 h/d over a 10-week period, achieving 98±67% average increase of the breast volume at the end of the expansion treatment, and 55% (range, 15–115%) at 30 weeks. The authors also reported very high patient satisfaction, no adverse events, and described the device as comfortable to wear.

However, after the enthusiasm generated by this first investigations, following researches outlined the limitations of the procedure: only small breast-size enlargement (1 cup) possible, high patient compliance required, patient social life restriction and drop out rates around 25%, 50% of the volume increase only due to swelling at 10 weeks with the suggestion to wear the device for 16–20 weeks.[12,22,35–38]

Despite the consequent modest success as nonsurgical breast augmentation procedure, the ability of Brava to determine a marked temporary increase in breast size with the creation of a very large fibrovascular scaffold induced several authors to investigate its potential as device to prepare the recipient site in fat grafting procedures.[12] These studies reduced the duration of the original protocol, with external volume expansion evolving from 2 to 3 months of static low pressure to 3 weeks individualized programs.[17]

Del Vecchio and Bucky[17] reported 5 main reasons supporting the use of Brava before fat grafting: (1) creation of more overall parenchymal space; (2) reduction of interstitial pressure in the breast for a given volume of transplanted graft; (3) modification of breast shape through augmentation of contour irregularities before grafting; (4) possibility to avoid variables such as high-speed centrifugation with resulting shorter operating room times; (5) angiogenesis, consequence of micromechanical forces on the recipient site.

Table 2. Complications Observed with the Use of Brava System and Fat Grafting

Complications	No. Instances (%)
Localized edema	181 (14.2)
Temporary bruising and superficial blistering	144 (11.3)
Fat necrosis	105 (8.2)
Erythema	18 (1.4)
Ulceration necrosis	18 (1.4)
Infection	16 (1.3)
Pruritus	14 (1.1)
Dysesthesia	14 (1.1)
Microcalcification	7 (0.5)
Pneumothorax	6 (0.5)
Oil cysts	5 (0.4)
Pseudocysts	3 (0.2)
Sleeping troubles	2 (0.2)
Phlyctena	1 (0.1)

Del Vecchio and Bucky[17] also noted that the degree of physical expansion obtained with Brava is not merely depending on the compliance of the patient, but also on the mechanical compliance of the recipient site. Indeed, they reported that multiparous breasts expanded better than dense nulliparous breast, while constricted breasts expanded well but required additional treatments such as nipple-areola reductions, percutaneous release of constriction bands to lower the inframammary folds, or additional grafting session to reshape the breasts. Moreover, Kosowski et al.[21] observed that irradiated tissue required more sessions of fat transplantation in comparison to nonradiated tissue to achieve satisfactory results, whereas Uda et al.[19] observed little skin extension in irradiated breast-conserving cases, resulting in poor cosmetic scores. Finally, regarding recipient sites characterized by contracted scar tissue, Uda et al.[19] reported higher improvement in case of total mastectomy compared with breast-conserving surgery, also in terms of total aesthetic score.

Recipient site features did not only impact the degree of expansion but also the complication rate. It was indeed observed a higher rate of complications in case of skin-sparing and nipple-sparing mastectomies, explained with the difficult release of folds and adhesions to the chest wall of mastectomy skin flaps and skin excess.[21] A high rate of skin complications was also reported in case of use of Brava on irradiated breast, as radiation therapy causes thinning of the epithelial tissue, affects blood circulation in the dermal tissue, and decreases dermal appendages, therefore inhibiting the regenerative ability of the skin.[19,39] Specifically, the ulceration rate was significantly larger in radiated breasts (6.5%) than in nonradiated breasts (1.4%) for postmastectomy patients in the study by Kosowski et al.[21]

However, although Uda et al.[19] discouraged the use of Brava on irradiated tissue for the above-mentioned reasons, Kosowski et al.[21] endorsed its use postulating that fat grafting to the irradiated breast could reverse radiation damage to yield superior results.[40] Yet, also Kosowski et al.[21] outlined that radiated breast tissue is less compliant, with consequent overgrafting and its inherent complications more likely to occur, recommending a greater craftsmanship and experience for a safe and effective execution of the procedure, and performance of multiple treatments (> 4) with small volumes of fat grafting.[21]

Notably, Hammer-Hansen et al.[20] emphasized the relevance of the dermatological side effects of Brava. They urged studies documenting the extent of this problem to provide clinical guidance for future use of the device and thus minimize or completely avoid these complications in future patients. Our review identified and quantified skin side effects of Brava as follows: temporal bruising and superficial blistering, 11.3%; erythema, 1.4%; ulceration necrosis, 1.4%; pruritus, 1.1%; phlyctena, 0.1%.[11,18,20,21]

Pneumothorax, the most serious complication observed, was reported by 2 authors who analyzed 427 and 476 patients, respectively, and affected 6 patients (overall, 0.5%), one of which required chest tube.[14,21]

Among the advantages of Brava, the authors emphasized that preexpanded breasts accept a greater fat transplant volume per session with a higher retention rate, resulting in less necessary session to achieve satisfactory results.[14,21] Indeed, we found 3 papers reporting significant enhancement of fat graft survival compared with fat grafting without preexpansion,[11,12,14] leading Khouri et al.[12] to state that if Brava is not used, the patient should accept smaller volume of fat grafting and multiple sessions, or life time implants in the aesthetic setting. We found a mean fat graft survival ranging between 53% and 82%.[11–14] In this regard, although we believe that the most meaningful outcome measure in fat grafting is percent augmentation instead of percent survival, as suggested by Khouri and Khouri[41] in 2015, we here refer to percent survival as this was the variable reported in the studies included in this review. The large amount of fat transplant possible after the use of Brava allowed total breast reconstruction and breast augmentation with satisfactory results. In fact, 7 authors performed megavolume fat transplant (≥ 300 cc),[2,7,11,13–15,17] and almost all the patients (1,272 over 1,274) received an average of more than 200 cc of fat graft per session.

Moreover, Kosowski et al.[21] observed that in contrast to traditional methods (implants and flaps), the use of Brava and autologuous fat transplantation holds the benefit to preserve or restore sensation of the breasts. However, our review also observed a rate of dysesthesia equal to 1.1%.[11]

Finally, our group described the use of an alternative device named Kiwi VAC-6000M with a PalmPump, for specific indications.[24] This was reported as a simple intraoperative external expansion system, which applies the cycling negative pressure of -550 mm Hg for 5 minutes to enhance small-volume AFG (40–80 mL). The rationale of preparing the recipient site with Kiwi is to obtain the release of contracted scar tissues by exploiting its traction force, and to promote intense edema, ischemia, and inflammation providing an ideal environment for cell proliferation and angiogenesis. The technique was described as especially useful in case of restrictive subdermal cicatrix, through the creation of a vascularized scaffold that is seeded with fat grafts, and in case of retractions or scarring as a result of radiation therapy.

CONCLUSIONS

Overall, positive outcomes were demonstrated with the use of external expansion in all the articles identified for both aesthetic and reconstructive purposes. Indeed, by allowing megavolume fat transplantation (> 300 cc), it appeared to be a valid alternative to implants for breast augmentation, and to free flaps and implants for breast reconstruction after total mastectomy. However, the low level of evidence of the studies that was found to be equal to maximum 3 in prospective case series, demonstrated the need of further exploring this topic.

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2.3. **Manuscript 3**

A Simple, Reliable, and Inexpensive Intraoperative External Expansion System for Enhanced Autologous Structural Fat Grafting.

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Authors: Oranges CM, Tremp M, Ling B, Wettstein R, Largo RD, Schaefer DJ.

Abstract - External volume expansion of the recipient site by suction has been proposed as a way of improving fat graft survival. The objective of this study was to present an innovative and simple intraoperative external expansion system to enhance small-volume autologous fat grafting (40-80 mL) and to discuss its background and its mechanism of action. In this system, expansion is performed using a complete vacuum delivery system known as the Kiwi VAC-6000M with a PalmPump (Clinical Innovations). The recipient site is rapidly expanded intraoperatively 10 times for 30 seconds each with a negative pressure of up to 550 mm Hg before autologous fat injection. During this repetitive stimulation, the tissues become grossly expanded, developing macroscopic swelling that regresses slowly over the course of hours following the cessation of the stimulus. The system sets various mechanisms in motion, including scar release, mechanical stimulation, edema, ischemia, and inflammation, which provide an environment conducive for cell proliferation and angiogenesis. In order to maintain the graft construct in its expansive state, all patients are encouraged postoperatively to use the Kiwi three times daily for one minute per session over the course of three days. The handling of this system is simple for both the patients and the surgeon. Satisfactory clinical outcomes have been achieved without significant complications.

Authorship statement: CMO and MT have a shared first authorship. CMO and MT both reviewed the literature, collected the data, wrote and corrected the paper. As corresponding author, CMO took care of the submission and revision process of the manuscript.

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A Simple, Reliable, and Inexpensive Intraoperative External Expansion System for Enhanced Autologous Structural Fat Grafting

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Idea and Innovation

External volume expansion of the recipient site by suction has been proposed as a way of improving fat graft survival. The objective of this study was to present an innovative and simple intraoperative external expansion system to enhance small-volume autologous fat grafting (40–80 mL) and to discuss its background and its mechanism of action. In this system, expansion is performed using a complete vacuum delivery system known as the Kiwi VAC-6000M with a PalmPump (Clinical Innovations). The recipient site is rapidly expanded intraoperatively 10 times for 30 seconds each with a negative pressure of up to 550 mm Hg before autologous fat injection. During this repetitive stimulation, the tissues become grossly expanded, developing macroscopic swelling that regresses slowly over the course of hours following the cessation of the stimulus. The system sets various mechanisms in motion, including scar release, mechanical stimulation, edema, ischemia, and inflammation, which provide an environment conducive for cell proliferation and angiogenesis. In order to maintain the graft construct in its expansive state, all patients are encouraged postoperatively to use the Kiwi three times daily for one minute per session over the course of three days. The handling of this system is simple for both the patients and the surgeon. Satisfactory clinical outcomes have been achieved without significant complications.

Keywords Subcutaneous tissue / Adipose tissue / Graft survival / Vacuum / Contracture / Cicatrix

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INTRODUCTION

Autologous fat grafting is becoming increasingly popular for soft tissue augmentation and reconstruction, due to improvements in donor site preparation and fat tissue harvesting, processing, and transplanting techniques. Moreover, adipose tissue demonstrates a regenerative potential related to the presence of mesenchymal stem cells [1]. However, the unpredictability of volume enlargement and fat tissue retention continue to pose major problems. Recent studies have investigated the role of the recipient site and its preconditioning in order to address these concerns [2,3].

Release of the contracture at the time of fat grafting is considered necessary in cases of contracted scar tissue, as seen in breasts with previous infection, radiation scarring, scar contracture from previous surgery, or congenital constriction bands, as seen in tuberous breasts [4]. "Rigottomies," named after Gino Rigotti, who described the technique, are commonly used to release and

stretch-expand scars by creating multiple tiny nicks inside the contracted tissue [4]. This technique transforms the scar into a three-dimensional mesh, expanding the volume of the recipient bed, and thereby reducing the interstitial fluid pressure into the adequate range for graft survival [4]. Attention is paid in order to avoid the creation of cavities [4].

Furthermore, the efficacy of pre-expansion external vacuum devices has been investigated in clinical and pre-clinical studies, especially to allow mega-volume (250-mL range) fat grafting in and around the breast [5]. The Brava system, a bra-like external expansion device, has been successfully used by Del Vecchio and Bucky [6] to precondition the recipient site and obtain a 60%–200% increase of human breast volume after autologous fat injection, as documented by quantitative magnetic resonance imaging. Moreover, Heit et al. [2] and Lancerotto et al. [3] demonstrated that external volume expansion applied to mouse integument induces considerable proliferation and vascularization in the subcutaneous tissue, which can be elicited even by a single two-hour external volume expansion cycle.

IDEA

Based on the above-discussed research, we have developed a new method to precondition localized scarred recipient sites intraoperatively in a simple, time-saving, and inexpensive manner. In order to obtain a combination of advantages similar to those described for Rigottomies and for preoperative external expansion systems, we apply an intraoperative external expansion device to prepare the recipient site for small-volume autologous fat grafts (40–80 mL) when the recipient site is characterized by a restrictive cicatrix or pre-irradiated tissues.

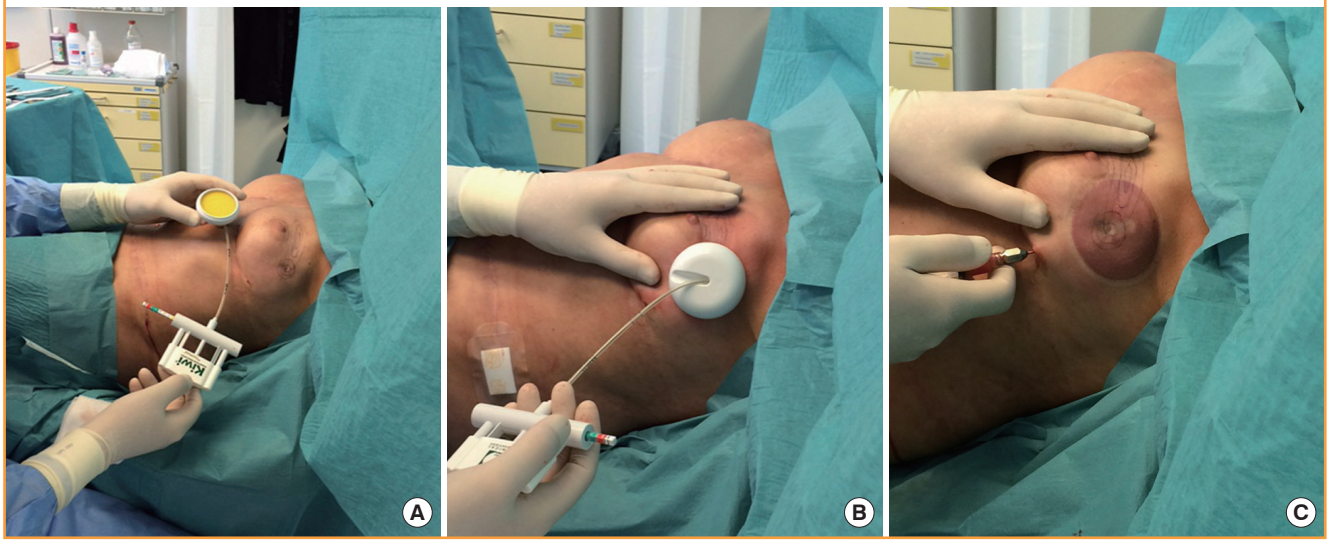
Expansion is performed using a complete vacuum delivery system known as a Kiwi VAC-6000M with a PalmPump (Clinical Innovations, South Murray, UT, USA). This vacuum delivery device is an integrated unit, providing an ergonomic handle with a simple hand-vacuum pump, thumb- or finger-activated vacuum release valve, and accurate vacuum indicator (Fig. 1). The device is relatively cheap (62 EUR) and readily available. It is used by gynecologists to extract fetuses. The recipient site is rapidly expanded intraoperatively 10 times for 30 seconds each with a negative pressure of up to 550 mm Hg before autologous fat injection (Fig. 2A, B). During this repetitive stimulation, macroscopic swelling of the soft tissue is observed (Fig. 2C). The re-

Fig. 1. The external expansion device

The complete vacuum delivery system used: Kiwi VAC-6000M with a PalmPump (Clinical Innovations).

Fig. 2. Intraoperative use of Kiwi on scar tissues

(A, B) The Kiwi is applied on a scarred pre-irradiated breast. (C) During the repetitive stimulation, macroscopic swelling of the soft tissue is observed.



cipient scaffold for fat grafts is then loosened and stretched out. This repetitive cycle leads to intense edema, ischemia, and inflammation, and provides an ideal environment for cell proliferation and angiogenesis.

In order to maintain the graft construct in its expansive state and further optimize the ideal graft-to-recipient volume ratio, all patients are encouraged to use the Kiwi postoperatively three times daily for a period of one minute per session over the course of three days.

The procedure can be performed under local or general anesthesia and, in our experience, has minimal morbidity and high patient acceptance and compliance. Patients who undergo local anesthesia for the procedure tolerate the device well and report only some moderate discomfort and pressure during application. The handling of the device is simple for both the patients and the surgeon. Postoperatively, some patients experience a small degree of edema, which normally resolves without sequelae.

DISCUSSION

Extreme variability is found in the literature regarding the ideal negative pressure to be applied on the recipient site prior to fat grafting. Several studies have investigated the effects of a negative pressure of -25 mm Hg applied for a single round of two hours or for two hours daily for five days in murine models, resulting in cell proliferation, neoangiogenesis, and neoadipogenesis [1,2,7].

A lower pressure (-125 mm Hg) was applied by Lee et al. [8] in an experimental model involving white rabbits. This negative pressure was continuously applied for one week prior to fat grafting. Lee et al. [8] reported improved recipient site vascularization and, accordingly, enhanced fat graft survival.

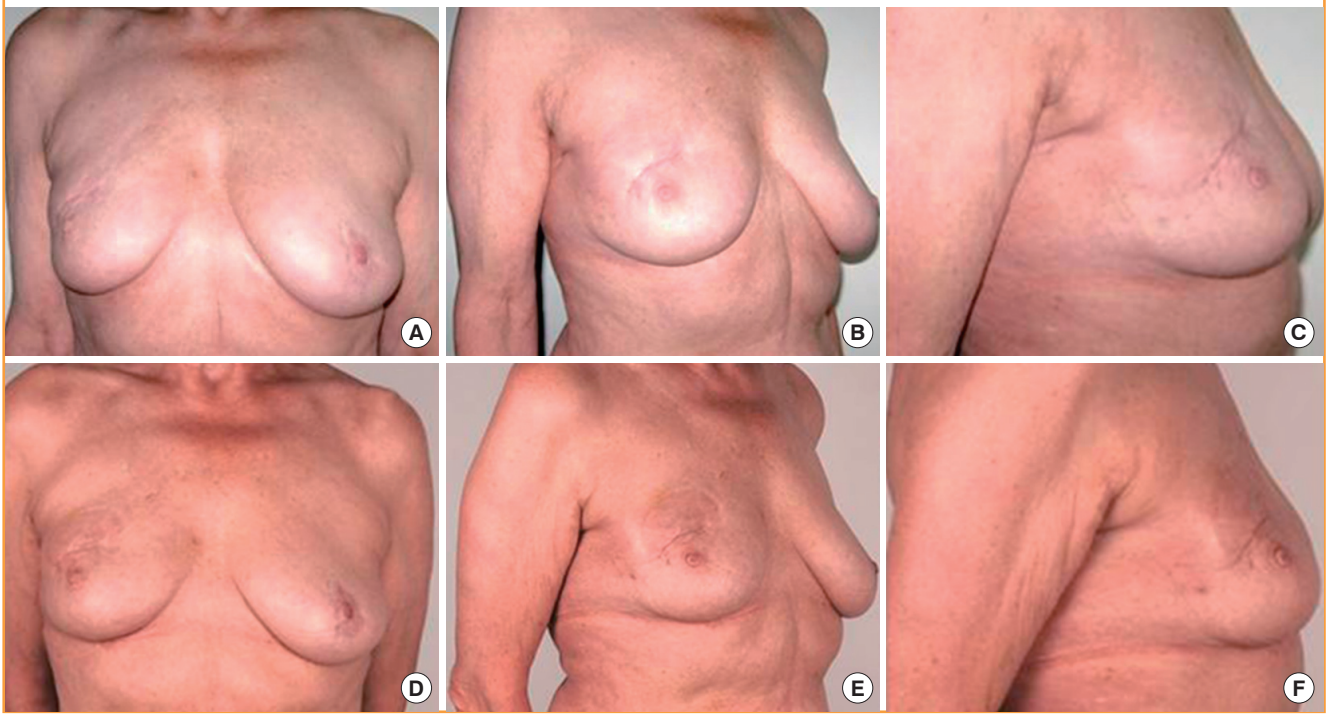
Finally, in their most recent clinical study of the Brava system, Khouri et al. [9] applied a high-vacuum cycling pump for 10 hours per day with a negative pressure of -60 mm Hg for two to four weeks prior to fat grafting.

In comparison with these reports from the recent literature, we apply a stronger negative pressure (-550 mm Hg) for a much shorter intraoperative period (five minutes). Following the recommendations of Chin et al. [10] and similarly to Khouri et al. [9], we apply cyclical forces to guarantee a more robust response, with the pump cycling between -550 mm Hg for 30 seconds to no pressure.

The rationale of using Kiwi prior to fat grafting is to obtain the release of contracted scar tissues by exploiting its traction force, and to promote intense edema, ischemia, and inflammation in

Fig. 3. Preoperative and postoperative views

(A-C) Preoperative views of a 67-year-old woman with a complaint of contracted scar tissue on the right breast, two years after breast-conserving surgery and radiation therapy. (D-F) Postoperative views seven days after one fat grafting procedure, with 40 mL grafted after recipient site preconditioning with Kiwi. Release of the scar with volume restoration is visible, although slight edema may be noted in the early follow-up images.



order to provide an ideal environment for cell proliferation and angiogenesis.

We hypothesize that, due to micromechanical strain, the application of pressure to the extracellular matrix results in an increase of cell anchorage to focal adhesion sites of the extracellular matrix fibers, although we are not able to characterize these events at the molecular level. These effects have largely been shown *in vitro*, where a proliferative state is induced by acting on a gate-control signal on global cellular activity mediated by the cytoskeleton [2,3].

The short-term use and application of high intraoperative pressure in a limited area may lead to results comparable to those obtained through the long-term use of the device, clinically confirming the experimental observations of Lancerotto et al. [3]. Our technique may be especially useful in cases of restrictive subdermal cicatrix, through the creation of a vascularized scaffold that is seeded with fat grafts. Conversely, it has been shown that the long-term use of pre-expansion has limitations, especially in severely deformed breasts after conservative surgery and radiation therapy, where it may induce skin complications such as dermatitis, pigmentation, or blistering.

In conclusion, based on our experience, we propose that this device is useful for preparing the recipient site for fat grafting, especially in cases where the surgeon must deal with retractions or scarring as a result of radiation therapy (Fig. 3).

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Three-dimensional Assessment of the Breast: Validation of a Novel, Simple and Inexpensive Scanning Process.

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Abstract - *Background/Aim:* Methods to assess threedimensionally the breast surface are increasingly used in plastic and reconstructive surgery. The aim of this study was to validate the use of the Structure Sensor 3D scanner (Occipital, Inc., Boulder, CO, USA) connected to an iPad Pro (Apple, Inc., Cupertino, CA, USA) as a novel, inexpensive and handheld three-dimensional scanning process. *Materials and Methods:* Surface images of a medical human female anatomy torso model of rigid plastic were repeatedly acquired with Structure Sensor 3D scanner and compared with those obtained using two clinically established 3D imaging systems. Digital measurements of vector and surface breast distances were analyzed using Mimics® Innovation Suite 20 medical imaging software (Materialise, Leuven, Belgium). *Results:* The analysis of variance (ANOVA) revealed no statistically significant difference among measurements obtained using different scanning processes for all the variables examined ($p>0.05$). *Conclusion:* The study demonstrates analogous practicability and reliability for surface image acquisition using the newly introduced Structure Sensor 3D scanner and other clinically established scanners.

Authorship statement: CMO is the first author. He designed the study, collected, analyzed and interpreted the data, wrote the manuscript and made tables. As corresponding author, CMO took care of the submission and revision process of the manuscript.

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Three-dimensional Assessment of the Breast: Validation of a Novel, Simple and Inexpensive Scanning Process

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Abstract. *Background/Aim: Methods to assess three-dimensionally the breast surface are increasingly used in plastic and reconstructive surgery. The aim of this study was to validate the use of the Structure Sensor 3D scanner (Occipital, Inc., Boulder, CO, USA) connected to an iPad Pro (Apple, Inc., Cupertino, CA, USA) as a novel, inexpensive and handheld three-dimensional scanning process. Materials and Methods: Surface images of a medical human female anatomy torso model of rigid plastic were repeatedly acquired with Structure Sensor 3D scanner and compared with those obtained using two clinically established 3D imaging systems. Digital measurements of vector and surface breast distances were analyzed using Mimics® Innovation Suite 20 medical imaging software (Materialise, Leuven, Belgium). Results: The analysis of variance (ANOVA) revealed no statistically significant difference among measurements obtained using different scanning processes for all the variables examined ($p>0.05$). Conclusion: The study demonstrates analogous practicability and reliability for surface image acquisition using the newly introduced Structure Sensor 3D scanner and other clinically established scanners.*

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Key Words: 3D scanning, three-dimensional, surface imaging, photogrammetry, virtual surgical planning.

Three-dimensional photographic imaging technologies are gaining an increasing role in plastic and reconstructive surgery. They allow accurate and efficient pre-operative analysis to formulate diagnosis and establish endpoint goals of treatment to address the underlying morphology and, thus, prepare an appropriate surgical plan (1).

Furthermore, in contrast to traditional two-dimensional photography, three-dimensional imaging is growingly regarded as a fundamental tool to objectively measure outcomes by providing true surface anatomy (2). Notably, volumetric and geometric parameters analyses such as depth and surface topographic distance measurements can also be performed, yielding important additional data (2).

These elements are considered particularly relevant in case of breast surgery, where they can be used to evaluate symmetry, surface and volumetric changes, including total breast volumes, volumetric distribution, and breast projection. Moreover, surface and vector measurements can be assessed to define breast contour, size, and position on the chest wall (3).

A number of researches have indeed validated the use of three-dimensional imaging in the clinical context of autologous or prosthetic breast reconstruction, and breast augmentation and reduction (3-10). Several validated devices are available for three-dimensional breast images capturing. However, many of them are not portable, heavyweight and generally expensive (11). These characteristics make them of unpractical use in the daily practice.

We previously described the use of a novel, simple and inexpensive three-dimensional scanning system for breast surface evaluation (12). The aim of this study was to validate its use in comparison to other clinically established three-dimensional imaging systems.

Materials and Methods

We performed a scanning process using the Structure Sensor 3D scanner (Occipital, Inc., Boulder, CO, USA) connected to an iPad Pro (Apple, Inc., Cupertino, CA, USA), available at a price of 379 USD. The device is a structured/infrared light handheld scanner that measures 11.92 (width) ×2.9 (height) ×2.8 (depth) cm and has a weight of 95 g.

The established Vectra M5 Scanner (Canfield Scientific Inc., Parsippany, NJ, USA) 3D imaging system and Artec Eva 3D scanner (Artec3D, Luxembourg, Luxembourg) were used as a reference. The former is a stationary passive stereophotogrammetry-based system, while the latter is a handled structured light three-dimensional scanning system that measures 26.1 (width) ×15.8 (height) x 6.3 (depth) cm and has a weight of 850 g. The last two devices are marketed at a higher price of over 10,000 USD.

The tree-dimensional scans were acquired, with all systems, on a medical human female anatomy torso model of rigid plastic in a room with normal illumination. The torso was scanned five times with each device. All scans were imported into the Mimics® Innovation Suite 20 medical imaging software (Materialise, Leuven, Belgium) to obtain from the three-dimensional images the following clinical measurements of each breast: sternal notch-to-nipple distance (S-N); nipple-to-inframammary fold distance (N-I); lateral inframammary fold-to-medial inframammary fold distance (L-M); upper pole-to-inframammary fold distance (U-I). All these distances were calculated both as a surface measurement and as a direct vector measurement. The vector distance between the two nipples (N-N) was also measured.

Furthermore, a single computer tomographic (CT) scan of the torso was also acquired and analyzed through the Mimics® Innovation Suite 20 medical imaging software (Materialise, Leuven, Belgium) as objective reference of the actual values of the measurements.

The analysis of variance (ANOVA) test was performed to determine if the differences among measurements obtained using different scanning processes were significant. Statistical significance was defined as $p<0.05$. All statistical analyses were performed using the SPSS® Advanced Statistical TM software package (ver. 13; SPSS Inc., Chicago, IL, USA).

Results

We calculated the landmark-based vector and surface distances. No statistically significant difference ($p>0.05$) was found with regard to all mean distances measured on the three-dimensional images captured using Structure Sensor 3D scanner, Vectra M5 scanner or Artec Eva 3D scanner. Moreover, no statistically significant difference was found in comparison to the images obtained using the CT scanner ($p>0.05$), with the only exception of the N-I surface distance of the left breast.

In detail, the following results were observed with regard to vector distances: mean L-M distance was equal in the right and left breast for each scanner with the exception of the CT scan, and ranged between 129.56 mm (SD=0.40) measured with Vectra M5 scanner and 130.07 mm (SD=1.34) measured with Artec Eva 3D scanner, with no statistical difference ($p=0.77$ in the right breast and $p=0.75$ in the left breast); mean S-N distance ranged between 179.07 mm (SD=1.06) and 179.77 mm (SD=0.78) in the right breast ($p=0.43$) and between 175.97 mm (SD=0.85) and 176.41 mm (SD=1.22) in the left breast ($p=0.67$); mean N-I distance ranged between 69.18 mm (SD=0.51) and 69.79 mm (SD=0.41) in the right breast ($p=0.35$) and between 69.45 mm (SD=0.38) and 70.03 mm (SD=0.83) in the left breast ($p=0.42$); mean U-I distance ranged between 167.64 mm (SD=0.68) and 168.11 mm (SD=0.24) in the right breast ($p=0.34$) and between 166.07 mm (SD=0.12) in the left breast ($p=0.20$). Finally, the mean N-N vector distance ranged between 195.94 mm (SD=0.44) and 196.57 mm (SD=1.88), with no statistically significant difference ($p=0.72$).

The detailed results regarding surface distances analysis are the following: mean L-M distance ranged between 187.81 mm (SD=1.25) and 189.30 mm (SD=0.99) in the right breast ($p=0.1$) and between 185.44 mm (SD=0.49) and 185.58 (SD=0.61) in the left breast ($p=0.8$); mean S-N distance ranged between 179.99 mm (SD=0.45) and 180.80 mm (SD=0.77) in the right breast ($p=0.1$) and between 178.96 mm (SD=0.81) and 179.26 mm (SD=0.99) in the left breast ($p=0.09$); mean U-I distance ranged between 196.87 mm (SD=0.35) and 197.15 mm (SD=0.30) in the right breast ($p=0.45$) and between 195.40 mm (SD=0.32) in the left breast ($p=0.70$). Finally, the mean surface N-I distance ranged between 69.43 mm (SD=0.31) and 69.73 mm (SD=0.44) in the right breast ($p=0.40$) and between 69.39 mm (SD=0.29) and 69.64 mm (SD=0.19) in the left breast ($p=0.39$), with no statistically significant difference among the Structure Sensor 3D scanner, Vectra M5 scanner or Artec Eva 3D scanner. However, in this last group, a statistically significant difference was observed with the CT scan images ($p=0.0004$).

Discussion

The application of three-dimensional imaging to breast surgery is a fast-developing concept that has been validated to date by a number of studies for both accuracy and reproducibility of the technology (1-9, 13-18). This has been considered particularly useful for the pre-operative planning and post-operative assessment of operations such as breast reconstruction, breast augmentation and breast reduction, as well as for the assessment of fat grafting outcomes where also small volume variations must be considered (3, 19-21). Our aim was to validate the use of an innovative and inexpensive scanning process.

In their comprehensive overview of the topic, Tepper *et al.* introduced the concept of mammometrics, defined as the establishment of fixed planes and points on three-dimensional

images to perform objective breast measurements (3). We calculated vectors and surface distances among landmarks of the breast and bony anatomical landmarks of the torso that represent important clinical measurements. The difference among the distances obtained using Structure Sensor and the established Vectra M5 scanner and Artec Eva 3D scanner, that was not found to be statistically significant, allowed us to validate the new scanning process.

The reasons for comparing Structure Sensor with Vectra M5 scanner and Artec Eva 3D scanner are based on the already validated use of these devices. In particular, Vectra technology has been used to capture face, neck, breast and body (13) while the clinical use of Artec Eva 3D has been described for the surface assessment of face, torso, upper and lower extremity (22-29). All three scanning processes showed consistent results also in comparison with the CT scanning, with only one exception, further demonstrating their accuracy.

A previous research compared the use of Structure Sensor and VECTRA®XT (Canfield Scientific, NJ, USA) 3D camera system, reaching the similar conclusion that Structure Sensor offers sufficient three-dimensional imaging quality to measure breast distances and volumes (11). In comparison with this research, which was performed in female breast patients, we decided to perform our measurements by using a medical human female anatomy torso model of rigid plastic in order to reduce the possible biases related to the need of ensuring the correct positioning of the patients or artifacts from highly moveable areas. Moreover, our results were also confronted with those obtained with Artec Eva 3D and CT scanners, ensuring a high level of objectivity.

The advantages of the use of Structure Sensor are certainly the portability, easy handleability and the low cost. However, both Vectra M5 and Artec Eva 3D solutions offer advanced software applications which are able to support the analysis of the three-dimensional images. For this purpose, the alternative use of an open access software such as MeshLab 2016 is possible but more complex and requires higher levels of training.

Overall, these findings are particularly important for their applications in the continuously evolving research in the field of breast and body contouring surgeries for both reconstructive and aesthetic purposes (30-36).

In conclusion, this research was able to validate the use of Structure Sensor 3D scanner (Occipital, Inc., Boulder, CO, USA) for breast surface assessment. However, further research in this area is needed to develop standardized procedures that can be used in the daily plastic and reconstructive surgery practice.

Conflicts of Interest

There are no conflicts of interest regarding this study.

Authors' Contributions

Study design: Carlo M. Oranges, Florian M. Thieringer, Philipp Brantner, Daniel F. Kalbermatten, Dirk J. Schaefer; Data acquisition and collection: Carlo M. Oranges, Florian M. Thieringer, Benito Benitez, Bilal Msallem; Statistical analysis: Carlo M. Oranges, Srinivas Madduri, Salvatore Giordano; Manuscript drafting: Carlo M. Oranges. Critical revision: all Authors. Final approval: all Authors.

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III. DISCUSSION

The objective of the present PhD thesis was to explore the preparation of the recipient site as a way of improving outcomes of AFG and to develop methods to assess results. In a first paper, we analyzed all existing literature on the studies which investigated different methods to prepare the recipient site prior to AFG in a pre-clinical setting, examining their mechanism of action and measurable endpoints. In a second manuscript, we analyzed all clinical studies on the preparation of the recipient site through pre-expansion, with resulting outcomes and possible complications. In a third article, we presented a novel external expansion method to prepare the recipient site using a device generating high cycling negative pressure, and discussed related indications, treatment protocol, and observed results. Finally, in a fourth paper we validated a novel, inexpensive and handled scanning process to evaluate breast volume and surface distances, with the aim of allowing objective measurements, particularly useful in case of AFG.

Overall, these four studies analyzed the best available evidence on the research in the field of the preparation of the recipient site in AFG and established innovative preparation and outcome assessment methods.

3.1 *Pre-clinical and clinical evidence on the preparation of the recipient site in fat grafting*

The success of the fat grafting procedure is substantially correlated to the characteristics and potential preparation of the recipient site (Rohrich and Pessa, 2007; Khouri RK et al, 2014; Del Vecchio and Del Vecchio, 2014; Khouri RK et al, 2014; Khouri RK Jr et al, 2014; Wang et al, 2017; Oranges et al, 2017). The first two publications included in this thesis provide a comprehensive analysis of methods used to prepare the recipient site based on both the pre-clinical and clinical evidence, with a special focus on external expansion.

Our review of the pre-clinical studies, revealed that a total number of 13 animal studies which applied five preparation techniques were published between 1992 and 2017.

We identified the following techniques: 1. external volume expansion; 2. implantation of alloplastic material (silicone sheets); 3. administration of cell-proliferating factors, namely vascular endothelial growth factor (VEGF), adipose tissue–derived stromal vascular fraction, and interleukin (IL)-8; 4. ischemia; 5. microneedling. In detail, external volume expansion was investigated in six animal studies on 122 mice, three pigs, and 20 rabbits (Giatsidis et al, 2017; Hsiao et al, 2016; Lujan-Hernandez et al, 2016; Lee et al, 2015; Lancerotto et al, 2013; Heit et al, 2012); implantation of alloplastic material in one animal study on 25 rabbits (Baran et al, 2002); administration of cell-proliferating factors in three animal studies (20 mice and 24 rats) (Topcu et al 2012; Koh et al, 2011; Shoshani et al, 2005); ischemia in one animal study (mice) (Gassman, Lewis and Lee, 2016); and microneedling in two animal studies on 38 rats. (Sezgin et al, 2014; Samdal et al, 1992).

The best evidence was found to be provided by the studies on controlled non-invasive suction, whose mechanism of action was therefore clarified. In particular, its application was able to determine a significant level of cell strain, ischemia, and edema (Giatsidis et al, 2017; Hsiao et al, 2016; Lujan-Hernandez et al, 2016; Lee et al, 2015; Lancerotto et al, 2013; Heit et al, 2012). These factors were correlated to the success of AFG due to their ability of triggering inflammation and lead to cell proliferation, angiogenesis, and adipogenesis. The impact of four pre-expansion variables was examined: the value of the negative pressure applied, the strength of cycling versus static pressures, the duration and the timing of the pre-expansion. There was no significant difference observed among these variables with the exception of the cyclical use of negative pressure which yielded a more robust response (Lee et al, 2015, Chin et al, 2010).

A positive effect on AFG was also shown with the use of alloplastic material implantation, namely delayed and reduced resorption of fat grafts. In this case, the mechanism of action was correlated to the foreign body reaction caused by the silicone sheets with a resulting chronic inflammatory response. The outcomes were therefore

explained with the longer time required by secondary wound healing compared with primary wound healing (Baran et al, 2002; Nguyen et al, 1990).

Improvement of fat graft survival was also obtained with the administration of cell proliferating factors, and associated to increased vascularity (blood vessel density) and cell proliferation. A further positive outcome was the reduction of cyst formation.

Remote ischemic preconditioning prior to AFG showed an increase in viability and substantially less interstitial fibrosis and liponecrosis in the pre-conditioned study group (Gassman et al, 2016). Gassman et al explained their findings with the improvement of distant capillary bed tissue oxygen saturation and perfusion, which limited ischemic injury to the transferred tissue.

Finally, regarding microneedling, a beneficial effect for fat transplantation was achieved through a higher level of vascularity expressed by higher blood vessel density, significantly less inflammation, cyst-vacuole formation, and fibrosis, according to the study by Sezgin et al (Sezgin et al, 2014). Overall, the mechanism of action of microneedling was therefore to induce a wound healing process through mechanical microtraumatization (Sezgin et al, 2014; Aust et al, 2011).

In summary, our review of the pre-clinical studies on the preparation of the recipient site in AFG clarified the mechanism of action of five techniques, explaining the outcomes of procedures already used clinically or offering the background for future clinical applications. Although achieved to different extents, all techniques showed positive outcomes in terms of fat graft survival, vascularity, cell proliferation, skin thickness, quality of tissue, and inflammation. However, with the exception of external volume expansion, whose use has already been widely applied in the clinical setting, the other techniques still require validation through future pre-clinical investigations and translation to clinics.

After the comprehensive analysis of the mechanisms of action, the second manuscript focused on reviewing all evidence provided by the clinical studies on the

preparation of the recipient site through external expansion. For the first time, all studies reporting outcomes and complications observed with pre-expansion performed for different indications and with different protocols were exhaustively examined.

Our inclusion criteria allowed the identification and final inclusion of 14 clinical papers, published from 2008 through 2016, describing the use of two external expansion devices, namely Brava system (Brava LLC, Miami, Fla.) and Kiwi VAC-6000M with a PalmPump (Clinical Innovations). With regard to the study design, we found 4 case reports, 6 retrospective, and 4 prospective studies. The maximum level of evidence was equal to 3 in prospective case series. Thirteen studies reported the treatment of 1,274 patients with the Brava system, while the single study that used Kiwi was published by our group and is discussed below in detail as part of this dissertation.

Brava was used in cases where the surgical indications for fat grafting were: breast reconstruction after mastectomy, aesthetic breast augmentation, correction of iatrogenic deformities emerging after excision of a congenital nevus or surgical cardiac procedure, correction of congenital deformities, and the desire of replacing pre-existing implants. The use of external expansion showed positive outcomes in all of the articles identified for both aesthetic and reconstructive purposes. In particular, one of the advantages was the ability of allowing megavolume fat transplantation (>250 cc), establishing AFG as valid alternative to implants for breast augmentation, and to free flaps and implants for breast reconstruction after total mastectomy. However, the use of the device was limited by the required significant degree of patient compliance and motivation, as Brava needed to be worn for 10 to 24 hours/day for 2 to 4 weeks pre-operatively according to recent published protocols, with a potential impact to patient social life and dropout rates.

Overall, the studies identified by our review confirmed the beneficial impact of using the Brava system before AFG, summarized by Del Vecchio and Bucky in 5 main points referred to the breast (Del Vecchio and Bucky, 2011): (1) increase of parenchymal space; (2) reduction of interstitial pressure for a given volume of fat graft; (3) modification of breast

shape to correct contour irregularities before grafting; (4) shorter operating room times by avoiding variables such as high-speed centrifugation; (5) induction of neo-angiogenesis as a consequence of the application of micromechanical forces.

However, despite the relatively large number of patients involved and the very promising results, the low level of evidence of the clinical studies implied the necessity of further researching the topic, possibly with randomized controlled trials comparing AFG with and without external volume expansion.

3.2. *The use of high cycling negative pressure in the preparation of fat grafting recipient site*

On the basis of the previous research discussed above, we developed and applied an innovative external expansion method to prepare the recipient site in case of localized restrictive cicatrix or pre-irradiated tissues. The new procedure was intended to be significantly simpler, time-saving and less expensive in comparison with the Brava system, although designed for different indications. This was the subject of our third manuscript.

The procedure used a complete vacuum delivery system called Kiwi VAC-6000M with a PalmPump (Clinical Innovations, South Murray, UT, USA). The external expansion was performed intraoperatively by generating a negative pressure of up to -550 mmHg before small-volume AFG (40–80 mL). The recipient site was rapidly expanded 10 times for 30 seconds each. This repetitive cycle generated macroscopic swelling of the soft tissue, loosened and stretched out the recipient scaffold, and leaded to intense edema, ischemia, and inflammation. An ideal environment for cell proliferation and angiogenesis was therefore observed. The use of Kiwi was also recommended during the first three post-operative days three times daily for a period of one minute per session, to maintain the graft construct in an expansive state and further optimize the ideal graft-to-recipient volume ratio in this very critical moment for graft intake.

Due to its use for localized conditions, the procedure could be performed under local anesthesia, while it was performed under general anesthesia if indicated by the overall patient conditions and surgical indications. The morbidity was minimal, with some patients presenting small degree of post-operative edema, normally resolving without sequelae. Conversely, the long-term use of Brava was associated to skin complications such as dermatitis, pigmentation, or blistering, particularly in severely deformed breasts after conservative surgery and radiation therapy (Uda et al, 2014).

Moreover, we observed high patient acceptance and compliance, which represented a further advantage in comparison with Brava, by definition offered to highly motivated patients willing to wear the device for several hours per day during the pre-operative weeks. The handling of Kiwi was also reported as simple for both patients and surgeon. The patients who underwent local anesthesia for the procedure tolerated the device well reporting only some moderate discomfort and pressure during application. Our clinical observations supported the idea that short-term use and application of high intraoperative pressure in a limited area can produce outcomes comparable to those obtained by using in the long-term devices generating low pressures, confirming previous experimental findings by Lancerotto et al (Lancerotto et al, 2013). In order to obtain an even stronger effect in a short time we also applied cyclical forces, as indicated by the research of Chin et al (Chin et al, 2010) and similarly to Khouri et al (Khouri, 2015), with the pump cycling between -550 mmHg for 30 seconds to no pressure.

However, although the use of Kiwi appeared very promising and with limited risk, further research will be required to further explore, validate and quantify the positive impact of using the procedure.

3.3 *Validation of a novel three-dimensional method to assess breast volumes and surface distances*

Assessment of AFG outcomes where also small volume variations must be considered require the use of sophisticated technologic devices. This is also generally required for objective pre-operative planning and post-operative assessment of operations such as breast reconstruction, breast augmentation and breast reduction (Tepper et al, 2010; Oranges et al, 2018; Oranges et al, 2016; Oranges and Schaefer, 2016). To this end, the use of three-dimensional imaging in breast surgery is rising and has now been validated for both accuracy and reproducibility of the technology by several studies (Losken et al, 2005; Kovacs et al, 2006; Isogai et al, 2006; Tepper et al, 2006; Tepper et al, 2008; Tepper et al, 2009; Tepper et al, 2010; Donfrancesco et al, 2013; Tzou et al, 2014; Chang et al, 2015; Epstein et al, 2015; Pfaff et al, 2016; Chae et al, 2016; Weissler et al, 2017; de Runz et al, 2018). The aim of our fourth manuscript was to validate the use of an innovative scanning process.

Our new system used the Structure Sensor 3D scanner (Occipital, Inc., Boulder, CO, USA) connected to an iPad Pro (Apple, Inc., Cupertino, Calif.). We compared its use with the already clinically established Vectra M5 (Canfield Scientific Inc., Parsippany, NJ, USA) and Artec Eva 3D (Artec3D, Luxembourg, Luxembourg) scanners. The Vectra technology has been used to capture face, neck, breast and body (Tzou et al, 2014), whereas Artec Eva 3D scanning system for surface assessment of face, torso, upper and lower extremities (Modabber et al, 2016; Yamamoto et al, 2016; Modabber et al, 2016; Seminati et al, 2017; Koban et al, 2018; Verhulst et al, 2018; Grant et al, 2019).

On the basis of previous research by Tepper et al which first described the concept of mammometrics, defined as the establishment of fixed planes and points on three-dimensional images to perform objective breast measurements (Tepper et al, 2010), we calculated and compared vectors and surface distances among anatomical landmarks of the breast and bony anatomical landmarks of the torso. The absence of statistically significant difference among these relevant clinical measurements obtained using the different methods

allowed the validation of the new scanning process. Moreover, the accuracy of the three scanning processes was also confirmed by the consistency of the data with those obtained using a Computed Tomography (CT) scanning process as gold standard.

Our results were congruous with previous research which also compared Structure Sensor 3D scanner and VECTRA®XT 3D camera system, reporting that Structure Sensor offered sufficient three-dimensional imaging quality to measure breast distances and volumes (Koban et al, 2018). However, we believe that our investigation was superior as the measurements were performed on a medical human female anatomy torso model of rigid plastic instead of female breast patients. This allowed to minimize biases related to the possibility of incorrect positioning of the patients or artifacts from highly moveable areas. Additionally, the high level of objectivity was also ensured by the further comparison with Artec Eva 3D and CT scanners.

The advantages of using Structure Sensor were certainly its portability, easy handleability and low cost. Indeed, it is available for 379 USD, can be easily connected to an iPad Pro, and has a weight of only 95 g. Instead, both Vectra M5 and Artec Eva 3D have a cost of over 10’000 USD, and are, respectively, the former a stationary passive stereophotogrammetry-based system and the latter a handled structured light three-dimensional device with a weight of 850 g.

Our findings support the use of this innovative tool, whose wide availability could overall facilitate the daily plastic surgery practice and the continuously evolving research in the field of breast and body contouring surgeries for both reconstructive and aesthetic purposes. It would be also particularly beneficial for objective AFG outcomes evaluation (Gabka et al, 1998; Munhoz et al, 2014; Amabile et al, 2015; Oranges et al, 2016; Gigli et al, 2017; Tremp et al, 2017; di Summa et al, 2019).

3.4. *Conclusion*

The preparation of the recipient site prior to fat grafting is a key aspect for the success of the procedure, mainly performed through pre-expansion. Based on the present work we propose the use of a novel pre-expansion device to be used in case of contracted scars or limited body contouring deformities. A novel three-dimensional scanning process has also been validated to measure breast volume and surface distances, allowing objective outcome evaluation for future investigations on fat grafting.

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V. CURRICULUM VITAE

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Date of birth: February 11th, 1983
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Languages: English, German, French, Italian.

Short Biography: skills and research interest

Dr. Carlo M. Oranges, MD, is a board certified plastic surgeon trained at the Basel University Hospital, Basel, Switzerland, and at the Marche Polytechnic University, Ancona, Italy. Dr. Oranges is a scholar in plastic surgery with over 70 peer-reviewed scientific publications indexed on PubMed/Medline. He is a member of the Swiss Society for Plastic, Reconstructive, and Aesthetic Surgery, the American Society of Plastic Surgeons (ASPS), the International Federation for Adipose Therapeutics and Science (IFATS), and the International Society of Regenerative Plastic Surgeons (ISPRES). His current area of research is the pre-conditioning of the recipient site in fat grafting.

Education and Training

PhD in Clinical Research	Department of Plastic, Reconstructive, Aesthetic, and Hand Surgery, University of Basel and Basel University Hospital, Basel, Switzerland (2016-2019).
Fellowship	Department of Plastic, Reconstructive, Aesthetic, and Hand Surgery, Basel University Hospital, Basel, Switzerland (March 2014 - June 2015, as a part of the residency program).
Residency	Marche Polytechnic University, Ancona, Italy - Plastic, Reconstructive, and Aesthetic Surgery School (May 2010 – May 2015).
Internship	S. Orsola – Malpighi University Hospital, Bologna, Italy - Division of Plastic Surgery (May 2008 – May 2010).
Interdisciplinary advanced education	Collegio Superiore Alma Mater Studiorum - School of Excellence of the University of Bologna, Bologna, Italy, providing advanced and interdisciplinary education (March 2003 – May 2008).
Medical School	Alma Mater Studiorum - University of Bologna, Bologna, Italy (September 2002 - May 2008). Doctor of Medicine (MD), full marks with highest honors. Title of MD dissertation: Stem Cells in Plastic Surgery: an Experimental Study on Adipose Tissue as a Resource for Regenerative Medicine.

Certifications/Licensures

Plastic, Reconstructive, and Aesthetic Surgery Board Certification.
Medical Licensure.

Memberships
Swiss Society of Plastic, Reconstructive, and Aesthetic Surgery (Swiss Plastic Surgery).
American Society of Plastic Surgeons (ASPS) – *International Member.*
International Society of Plastic Regenerative Surgeons (ISPRES).
International Federation for Adipose Therapeutics and Science (IFATS)

Award
ISPRES/ICOPLAST Young Plastic Surgeon Award, Dubai, UAE, November 18th, 2017.

Relevant Courses
Microsurgical Advanced Course, DAM Certification (German Speaking Society of Microsurgery)
Microsurgical Basic Course, DAM Certification (German Speaking Society of Microsurgery)

Editorial Activity
The Lancet (Reviewer)
Plastic and Reconstructive Surgery (Reviewer)
PRS Global Open (Reviewer)
Aesthetic Surgery Journal (Reviewer)
Journal of Plastic, Reconstructive and Aesthetic Surgery (Reviewer)
Dermatologic Therapy (Reviewer)

Visiting professorship
Turku University Hospital and the University of Turku, Turku, Finland (April 15th 2019, lecture about "Gluteal Augmentation Techniques).

Publications
Peer-Reviewed Journals

1. **Oranges CM**, Madduri S, Brantner P, Msallem B, Giordano S, Benitez B, Kalbermatten DF, Schaefer DJ, Thieringer FM. Three-dimensional Assessment of the Breast: Validation of a Novel, Simple and Inexpensive Scanning Process. In Vivo 2019 May-Jun;33(3):839-842.
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3. **Oranges CM**, Striebel J, Tremp M, Madduri S, Kalbermatten DF, Harder Y, Schaefer DJ. The Preparation of the Recipient Site in Fat Grafting: A Comprehensive Review of the Pre-Clinical Evidence. *Plast Reconstr Surg*. 2019 Apr;143(4):1099-1107.
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5. Sörelius K, Schiraldi L, Giordano S, **Oranges CM**, Raffoul W, di Summa PG. Reconstructive Surgery of Inguinal Defects: A Systematic Literature Review of Surgical Etiology and Reconstructive Technique. *In Vivo*. 2019 Jan-Feb;33(1):1-9.
6. di Summa PG, **Oranges CM**, Wafta W, Sapino G, Keller N, Tay SK, Chew BK, Schaefer DJ, Raffoul W. Systematic review of outcomes and complications in non-implant based mastopexy surgery. *J Plast Reconstr Aesthet Surg.* 2019 Feb;72(2):243-272.
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9. **Oranges CM**, di Summa PG, Kalbermatten DF, Haug M, Schaefer DJ. Staying Safe during Gluteal Fat Transplantation. *Plast Reconstr Surg*. 2018 Jul 16. doi: 10.1097/PRS.0000000000004786. [Epub ahead of print]
10. **Oranges CM**, Schaefer KM, Haug M, Schaefer DJ. The Impact of Labiaplasty on Sexuality. *Plast Reconstr Surg*. 2018 Jul 16. doi: 10.1097/PRS.0000000000004784. [Epub ahead of print]
11. **Oranges CM**, Ling B, Tremp M, Wettstein R, Kalbermatten DF, Schaefer DJ. Comparison of Anterolateral Thigh and Radial Forearm Free-Flaps in Head and Neck Reconstruction. *In Vivo*. 2018 Jul-Aug;32(4):893-897.

12. di Summa PG, Schiraldi L, Cherubino M, **Oranges CM**, Kalbermatten DF, Raffoul W, Madduri S. Adipose derived stem cells reduce fibrosis and promote nerve regeneration in rats. *Anat Rec* (Hoboken). 2018 Apr 30. doi: 10.1002/ar.23841. [Epub ahead of print].
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15. Thuerlimann A, Tremp M, **Oranges CM**, Schaefer DJ, Kalbermatten DF. Intentional Lower Pole Rotation of Anatomic Breast Implants in Chest Wall Deformities. *Plast Reconstr Surg Glob Open.* 2017;6:e1605.
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41. **Oranges CM**, Gohritz A, Tremp M, Schaefer DJ. Body Contouring: the Success of the Androgynous Model. *Plast Reconstr Surg Glob Open*. 2016 Apr 1;4(4):e668.
42. Sisti A, Sisti G, **Oranges CM**. Effectiveness and safety of topical tacrolimus monotherapy for repigmentation in vitiligo: a comprehensive literature review. *An Bras Dermatol*. 2016 Mar-Apr; 91(2): 187–195.
43. **Oranges CM**, Meier C, Schaefer DJ. Surgical Timing and the Menstrual Cycle Affect Wound Healing in Young Breast Reduction Patients. *Plast Reconstr Surg*. 2016 Sep;138(3):547e.
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52. **Oranges CM**. ENDOCRINOLOGY AND ART: Proportionate dwarfism in a painting of Rodrigo de Villandrando (1620). *J Endocrinol Invest*. 2015 Aug;38(8):929.
53. **Oranges CM**. Thoughts on "Commentary on Labia Minora Reduction Techniques: A Comprehensive Literature Review" *Aesthet Surg J*. 2015 Nov;35(8):NP270-1.
54. **Oranges CM**, Matucci-Cerinic M. Endocrinology and Art: Maddalena Ventura: an impressive case of hirsutism in a painting of Jusepe De Ribera (1631) *J Endocrinol Invest*. 2016 Jan;39(1):123.
55. Negosanti L, Sgarzani R, Fabbri E, Palo S, **Oranges CM**, De Iaco P, Zannetti G, Contedini F, Cipriani R. Vulvar Reconstruction by Perforator Flaps Algorithm for Flap Choice Based on the Topography of the Defect. *Int J Gynecol Cancer*. 2015 Sep;25(7):1322-7.
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57. **Oranges CM**, Sisti A. Medial Thigh Lift in the Massive Weight Loss Population. *Plast Reconstr Surg*. 2015 Aug;136(2):273e-274e.
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59. Sisti A, **Oranges CM**. Evidence-Based Medicine: Unilateral Cleft Lip and Nose Repair. Plast Reconstr Surg. *Plast Reconstr Surg*. 2015 Jul;136(1):118e-119e.
60. Sisti A, **Oranges CM**. Functional Considerations in Aesthetic Eyelid Surgery. *Plast Reconstr Surg*. 06/2015; 135(6):1066e.
61. Contedini F, Negosanti L, Pinto V, **Oranges CM**, Sgarzani R, Lecce F, Cola B, Cipriani R. Reconstruction of a complex pelvic perineal defect with pedicled anterolateral thigh flap combined with bilateral lotus petal flap: A case report. *Microsurgery*. 2015 Feb;35(2):154-7.

62. Sisti A, Sisti G, **Oranges CM**. Topical treatment of melanoma skin metastases with Imiquimod: a review. *Dermatol Online J*. 2014 Dec 12;21(2).
63. Tarsitano A, Sgarzani R, Betti E, **Oranges CM**, Contadini F, Cipriani R, Marchetti C. Vascular pedicle ossification of free fibular flap: is it a rare phenomenon? Is it possible to avoid this risk? *Acta Otorhinolaryngol Ital*. 2013 October; 33(5): 307–310.
64. Tarsitano A, Pizzigallo A, Sgarzani R, **Oranges CM**, Cipriani R, Marchetti C. Head and neck cancer in elderly patients: is microsurgical free-tissue transfer a safe procedure? *Acta Otorhinolaryngol Ital*. 2012 Dec;32(6):371-5.
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72. Chen G, **Oranges CM**, Giordano S, Huang R, Wang W. Horizontal Animation Deformity as Unusual Complication of Neurotoxin Modulation of the Gummy Smile in an Asian Patient: A Case Report. Accepted for publication in *Dermatol Online J.*

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Other publications

1. Morselli PG, Pinto V, Morellini A, Sgarzani R, Negosanti L, Tavaniello B, Faenza M, **Oranges CM** Rinocheilognatoschisi monolaterale: approccio globale alla dismorfia della punta nasale. – Monolateral rhinocheiloschisis: global approach to nasal tip dysmorphia - *Rivista Italiana di Chirurgia Plastica,* Vol. 40, 33-43, 2008.
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Abstract and Poster Presentations

1. **Oranges CM**, Tremp M, Wang W, Madduri S, di Summa PG, Wettstein R, Schaefer DJ, Kalbermatten DF. Patient Height, Weight, BMI and Age as Predictors of Gracilis Muscle Free-Flap Mass in Lower Extremity Reconstruction. World Society for Reconstructive Microsurgery (WSRM), Bologna, Italy, 12-15 June 2019.
2. **Oranges CM**, Tremp M, Wang W, Madduri S, di Summa PG, Wettstein R, Schaefer DJ, Kalbermatten DF. Patient Height, Weight, BMI and Age as Predictors of Gracilis Muscle Free-Flap Mass in Lower Extremity Reconstruction. EURAPS Research Council Annual Meeting, Helsinki, Finland, May 22-23, 2019.
3. **Oranges CM**, Madduri S, Brantner P, Msallem B, Giordano S, Benitez B, Kalbermatten DF, Schaefer DJ, Thieringer FM. Three-dimensional Assessment of the Breast: Validation of a Novel, Simple and Inexpensive Scanning Process. EURAPS Research Council Annual Meeting, Helsinki, Finland, May 22-23,

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4. **Oranges CM**, Tremp M, di Summa PG, Haug M, Kalbermatten DF, Harder Y, Schaefer DJ. Gluteal Augmentation Techniques: A Comprehensive Literature Review. The 18th International Congress of Plastic and Reconstructive Surgery in Shanghai-The 8th International Symposium on Plastic and Cosmetic Surgery in Shanghai, Shanghai, China, March 10-17 2019.
5. Kalbermatten DF, **Oranges CM**. The treatment of decubitus in Plastic Surgery. AAWC Pressure Ulcer Summit, Atlanta, GA, USA, February 8-9, 2019
6. **Oranges CM**, Harder Y, Haug M, Daniel F. Kalbermatten, Schaefer DJ, Thieringer FM. Three-Dimensional Evaluation of Breast Volumes: A Novel Approach to Assess Fat Grafting Outcomes. 16th Annual Meeting of the International Federation for Adipose Therapeutics and Science(IFATS), Las Vegas , Nevada, December 13 - December 15, 2018.
7. **Oranges CM**, Sisti G, Nasioudis D, Tremp M, di Summa PG, Kalbermatten DF, Largo RD, Schaefer DJ. Hard Palate Melanoma: A Population-based Analysis of Epidemiology and Survival Outcomes. 40. Jahrestagung der Deutschsprachigen Arbeitsgemeinschaft für Mikrochirurgie der Peripheren Nerven und Gefäße (DAM), Lugano, Switzerland, November 8-10, 2018.
8. **Oranges CM**, Tremp M, Ling B, Wettstein R, Largo RD, Schaefer DJ. A Simple, Reliable, and Inexpensive Intraoperative External Expansion System for Enhanced Autologous Structural Fat Grafting. ISAPS 2018 (International Society of Aesthetic Plastic Surgery), Miami Beach, Florida, United States, October 31th-Novemver 4th, 2018
9. **Oranges CM**, Tremp M, di Summa PG, Haug M, Kalbermatten DF, Harder Y, Schaefer DJ. Gluteal Augmentation Techniques: A Comprehensive Literature Review. Meeting of the European Society of Plastic, Reconstructive and Aesthetic Surgery (ESPRAS) 2018, Limassol, Cyprus, October 23-27, 2018.
10. **Oranges CM**, Harder Y, Haug M, Kalbermatten DF, Schaefer DJ. Efficacy and Safety of Gluteal Augmentation with Autologous FAT Grafting: A Systematic Review. 7th ISPRES (International Society of Plastic Regenerative Surgeons) Annual Meeting joint with Plastic Surgery The Meeting (American Society of Plastic Surgerons), Chicago, Illinois, USA, September 28 – October 1, 2018.
11. **Oranges CM**, Tremp M, Ling B, Wettstein R, Largo RD, Schaefer DJ. A Simple, Reliable, and Inexpensive Intraoperative External Expansion System for Enhanced Autologous Structural Fat Grafting. 7th ISPRES (International Society of Plastic Regenerative Surgeons) Annual Meeting joint with Plastic Surgery The Meeting (American Society of Plastic Surgerons), Chicago, Illinois, USA, September 28 – October 1st, 2018.
12. Schiraldi L, di Summa PG, Cherubino M, **Oranges CM**, Kalbermatten DF, Raffoul W, Madduri S. Adipose derived stem cells reduce fibrosis and promote nerve regeneration in rats. 54th Annual Meeting of Swiss Plastic Surgery joint with the 6th Congress of Swiss Aesthetic Surgery, Crans-Montana, Switzerland, 21-22 September 2018.
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17. **Oranges CM**, Striebel J, Tremp M, Madduri S, Kalbermatten DF, Schaefer DJ. The Impact of Recipient Site External Expansion in Fat Grafting Surgical Outcomes. 7th EURAPS Research Council, Madrid, Spain, May 16th-17th 2018.
18. Tremp M, Thuerlimann A, **Oranges CM**, Schaefer DJ, Kalbermatten DF. Intentional Lower Pole Rotation of Anatomic Breast Implants in Chest Wall Deformities. 7th EURAPS Research Council, Madrid, Spain, May 16th-17th 2018.
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20. Tremp M, Thuerlimann A, **Oranges CM**, Schaefer DJ, Kalbermatten DF. Correction of congenital thoracic wall deformities using breast implants – Reconstructive surgery meets aesthetic surgery. IMCAS Annual World Congress 2018, Paris, France, February 1-3, 2018.

21. Tremp M, Burger M, Kalbermatten DF, **Oranges CM**. Invited lecture: The Use of Platelet-Rich Plasma (PRP) in Aesthetic Surgery. Plastic and Reconstructive Surgery Department, Shanghai Ninth People's Hospital (Prof. Xiao-xi Lin), Shanghai, China, January 4, 2018.
22. **Oranges CM**, Striebel J, Tremp M, Kalbermatten DF, Schaefer DJ. The role of recipient site pre-conditioning techniques in fat grafting. Ninth International Conference on Regenerative Surgery, Rome, Italy, 15-16 December 2017
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25. Kalbermatten DF, **Oranges CM**. Tongue Reconstruction. 39. Jahrestagung der Deutschsprachigen Arbeitsgemeinschaft für Mikrochirurgie der Peripheren Nerven und Gefäße (DAM), Freiburg, Germany, November 16-18 2017.
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40. **Oranges C**M, Largo RD, Sisti G, Nasioudis D, Schaefer DJ. Epidemiology and Survival of Patients with Hard Palate Melanoma: a Population-based Study. EURAPS Research Council and European Plastic

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47. Morselli PG, Pinto V, **Oranges CM**, Bagnara GP, Bonsi L, Marini M, Marchionni C, Alviano F. How do you harvest fatty tissue? Does it make any difference on the outcome of stem cells growth and apoptosis? - International Symposium on Plastic Surgery – Bologna, June 25th - 28th, 2008.
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52. Pinto V, Tassone D, Negosanti L, Tavaniello B, Palo S, Nejad P, Zavalloni F, **Oranges CM**, Morselli PG. Madelung's disease: a case report. (6th SPIGC National Congress of Italian Surgery Residents, Forlì, Italy, 2010).
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Contedini F, Morselli PG, Marchetti C, Cipriani R. Lembi liberi microvascolari per la ricostruzione cervicofacciale: comparazione dei risultati oncologici e ricostruttivi fra pazienti giovani e anziani. - Microvascular free flaps in head and neck reconstruction. Comparison of oncologic and reconstructive outcomes in elderly and young patients. (60th Italian National Congress of SICPRE, Società Italiana di Chirurgia Plastica - Italian Society of Plastic Surgery, Milan, 2011).

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