Long Term Rejuvenation of Facial Skin by Transplantation of Autologous Stromal Vascular Fraction and Pre-conditioned Matrix: A Clinical Study

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Abstract

Background: For skin rejuvenation, today no procedure describes a specific treatment of the extracellular matrix (ECM) combined with stromal vascular fraction (SVF) from adipose tissue. A new product AmeaCell®, allows SVF extraction and ECM preconditioning in hypoxic conditions. Hypoxia significantly increased the adhesion of adipose derived stem cells and enhace their proliferation and differentiation.

Objective: This clinical study aimed to evaluate safety and efficiency of this new procedure, over one year.

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Material and methods: The study was performed on 23 patients. After a liposuction and fat proceed, SVF and the matrix were extracted and preconditioned following Ameacell® procedure, then injected into wrinkles. Results were scored for WSRS and GAIS scales at 6, 12 months after surgery.

Results: No serious adverse reactions occurred. Regarding efficiency, whatever the baseline, the Ameacell® procedure triggered an improvement of WSRS score in most patients after one year follow-up: 75% of patients when WSRS score at baseline 2; 83% of patients when WSRS score at baseline 3. This is in accordance with the GAIS scored by both investigators and patients.

Conclusion: This one-year follow-up clinical study using Ameacell® procedure, exhibited an improvement of facial skin appearance.

Keywords: Clinical study, SVF, stem cells, hypoxia.

Introduction

The stromal vascular fraction (SVF), harvested from human adipose tissue, is composed of numerous cell types such as endothelial progenitor cells, pericytes, pre-adipocytes, lymphocytes, monocytes, granulocytes and adipose derived stem cells (ADSC). ADSC exhibit anti-inflammatory and neoangiogenic effects and can differentiate into various cell types [1]. The use of SVF and ADSC have gained much importance since several years, for skin rejuvenation [2].

ADSCs have a broad paracrine action on dermal cells: angiogenesis stimulation, inflammation and immune tolerance modulations. Moreover, ADSC secretions induce dermal collagen synthesis by increasing fibroblasts and keratinocytes proliferation and activity, which counteract the effects of ageing [3]. ADSC could also be differentiated into epithelial lineage cells [4]. Klar suggested that endothelial progenitors among SVF, can differentiate and promote dermal vascularization [5].

During aging, inflammation leads to collagen degradation [6]. Among SVF, it has been reported that both immune cells, and ADSC exhibit anti-inflammatory effects [7] which can counteract collagen degradation.

The microenvironment of the skin in which SVF are injected, is represented by the extra cellular matrix (ECM). ECM is mainly composed of collagens, glycosaminoglycans, involved in cell adhesion, pre-requisite to cell proliferation and differentiation. The device AmeaCell® presented here,

allows, the treatment of this ECM by hypoxia (called MatriCS), and SVF extraction.

Hypoxic-treated ADSC have a low risk of genetic instability [8]. After SVF and MatriCS co-injection, immunohistological studies had exhibit cell proliferation and collagen synthesis through SVF differentiation into dermofibroblasts [9]. Moreover, cells co-injected with MatriCS, remained on the site of injection (personal communication).

The present clinical study evaluated safety and efficiency of SVF and MatriCS co-injection using AmeaCell® as a new procedure, for skin rejuvenation.

Materials and Methods

This study was a multicentric, open-label, non-randomized prospective clinical study. It evaluated the effects of SVF and MatriCS co-injection in the improvement of skin quality of 23 patients. After ensuring that inclusion and exclusion criteria were fulfilled (Table 1), patients gave written informed consent.

The follow-up, over one year, assessed the long-term safety of the product as the primary objective. Filling efficacy over the same period was the second objective. This research has been registered on Clinicaltrials.gov: NCT03529292. The study was conducted under the regulation 2016/679/EU on the protection of natural persons regarding the personal data and free movement of such data.

7 visits have been planned: screening visit (V0; from 60 to 15 days before surgery), inclusion visit (V1 "baseline"; from 15 to 2 days before filling),

Table 1 Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
♂ or ♀ 18 to 70 year old	♀ pregnant/lactating/planning a pregnancy
patient wishing to have a liposuction and a filling of facial wrinkles for aesthetic purposes.	Patient having received within the previous 12 months/planning to have a filling procedure whatever the type of lipofilling.
For whom it is possible to harvest at least 150 mL of liposuction.	Family history/background/allergic contact dermatitis.
WSRS grade ≥ 2 for at least 1 wrinkle.	Scar in treatment area that may interfere with assessment.
	Diabetes (type I or II)
	HBV, HCV, HIV positive or on HIV treatment
	Autoimmune disease/immunodepression

surgical intervention visit (V2: SVF and MatriCS co-injection, D0), and in post-surgery visits: V3 (D0 + 2 weeks), V4 (D0 + 1 month), V5 (D0 + 6 months), V6 (D0 + 12 months).

For safety assessment, side effects such as oedema, ecchymosis, bruising, and pain/tenderness were registered during 12 months after injection. For efficacy assessment, wrinkle depth was assessed by wrinkle severity rating scale (WSRS) at 1, 6, 12 months post-surgery and was compared to the baseline, scored from 0 (none) to 4 (severe). 2D and 3D pictures were taken with LifeViz Mini (Quantificare, France) to assess aesthetic improvements in addition to the scoring with global aesthetic improvement scale (GAIS) done by surgeons and by patients.

AmeaCell® device is composed of a kit of consumables and a dedicated equipment, (Symbioken, France).

Briefly, SVF and MatriCS were isolated from 100 mL of densified fat from subcutaneous adipose tissue as described previously [1]. The Coleman method has been used, then, densified fat was digested with collagenase. After centrifugation for 10 min at 300 g, SVF were pelleted, and the matrix was treated with a cobalt chloride solution to induce hypoxia, at 200 μM during 20 min under shaking and becomes MatriCS. At the end of the procedure, MatriCS and SVF were subcutaneously injected using a 16 gauge micro cannula to perform a diffuse distribution, into layer of the skin. The statistical significance of our data was performed by using SAS® software, and was accepted for p values < 0.05.

Results

Out of 23 patients 3 patients discontinued in context of Covid 19 pandemic, 2 patients didn't have enough fat after liposuction and a protocol deviation has been done for 3 patients since use of Adisculpt leads to a large loss of ECM. For the 15 patients that completed the study, no adverse event was classified of serious.

At 6 and 12 months after surgery, when the WSRS score was 2 at baseline, for the left nasolabial fold, 100% of patients improved (delayed improvement at 6 months for one patient) and was maintained at 1-year-follow-up period for 25% of patients. For the right nasolabial fold, the 100% of improvement was still observed after one year for 75% of patients.

When the WSRS score was 3 at baseline, the score for the left nasolabial folds improved for 88% of patients, and was still observed after one year for

71% patients. For the right nasolabial fold, the score improved for 86% of patients, and was still observed after one year for 83% of patients.

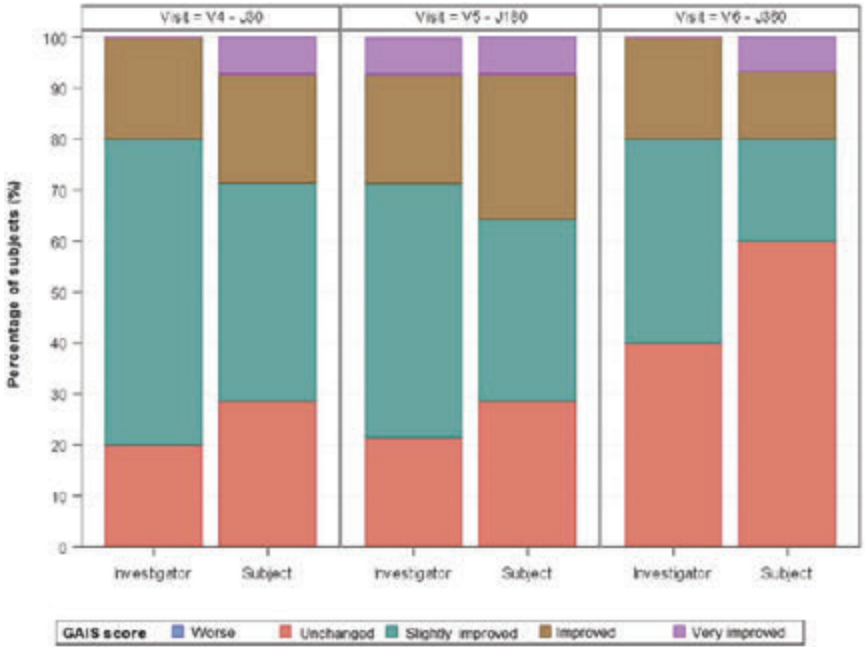
When the WSRS score was 4 at baseline, the score for the left and right nasolabial folds improved for 100% of patients and was still observed after one year for 50% of patients.

Whatever the score at baseline, SVF and MatriCS co-injection triggered an improvement of the WSRS score at 3 months and/or 6 months, in most patients. When the WSRS score is 4 at baseline, the improvement is scored in 100% of patients.

After one year follow-up, the improvement is maintained for patients who had an intermediate WSRS score at baseline, i.e, 3.

Regarding GAIS score, shown on Table 2, both investigators and patients were asked to independently evaluate the aesthetic improvement compared to the pre-treatment state. Most investigators and patients considered that the treatment according to AmeaCell® procedure triggered an aesthetic improvement.

Table 2 GAIS, percentage of investigators and patients who scored an aesthetic improvement at 1 month, 6 months and 12 months after the treatment



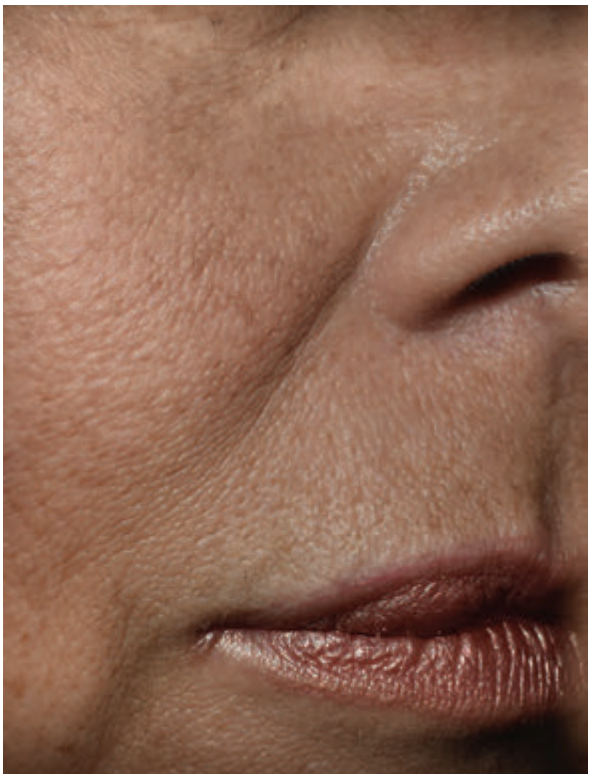


Figure 1 Patient 1, before injection.

The technology developed by Quantificare was used to assess wrinkle volume change. A 3D stereoscopic camera acquired a 3D representation of the face surface. Then the system is matching baseline and a follow-up 3D surfaces, to measure volume difference in the wrinkles. Most pictures were useless because patients adopted a slightly different position. For this reason, the change of volume of the nasolabial folds could not be analyzed in a meaningful way. Nevertheless, all data have been provided and some pictures has been extracted (Figures 1 to 6) showing skin improvement.

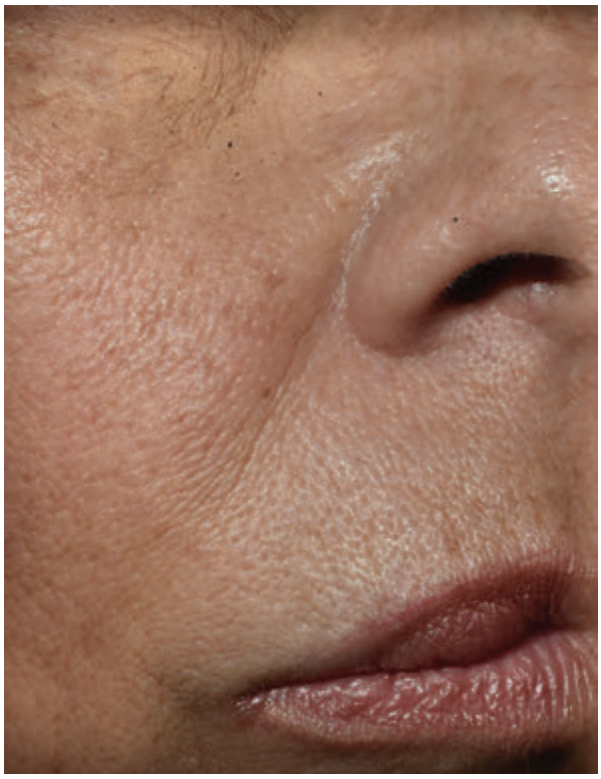


Figure 2 Patient 1, after injection, 1 year.

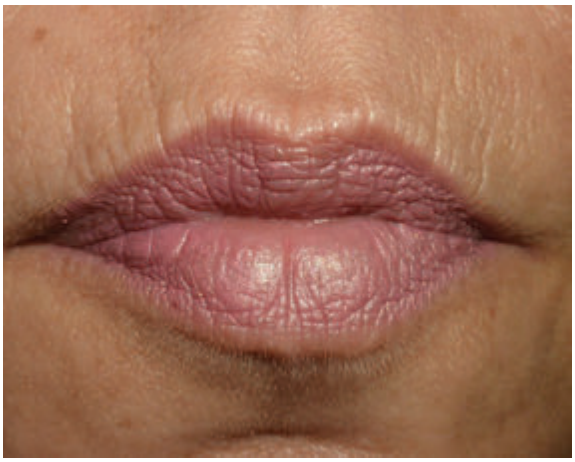


Figure 3 Patient 2, before injection.

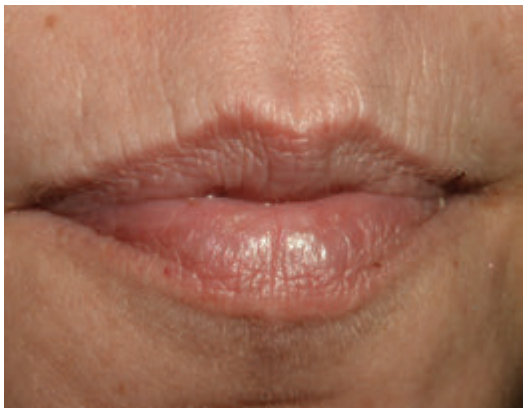


Figure 4 Patient 2, after injection, 1 year.

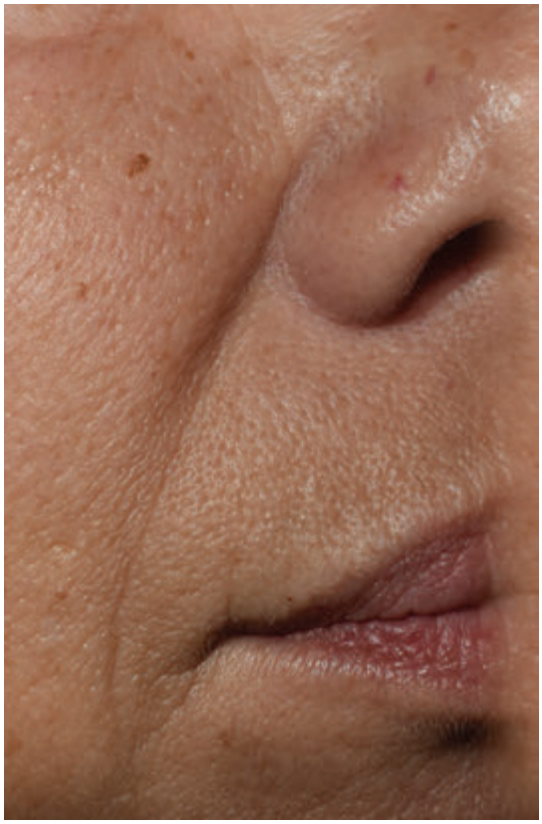


Figure 5 Patient 3, before injection.

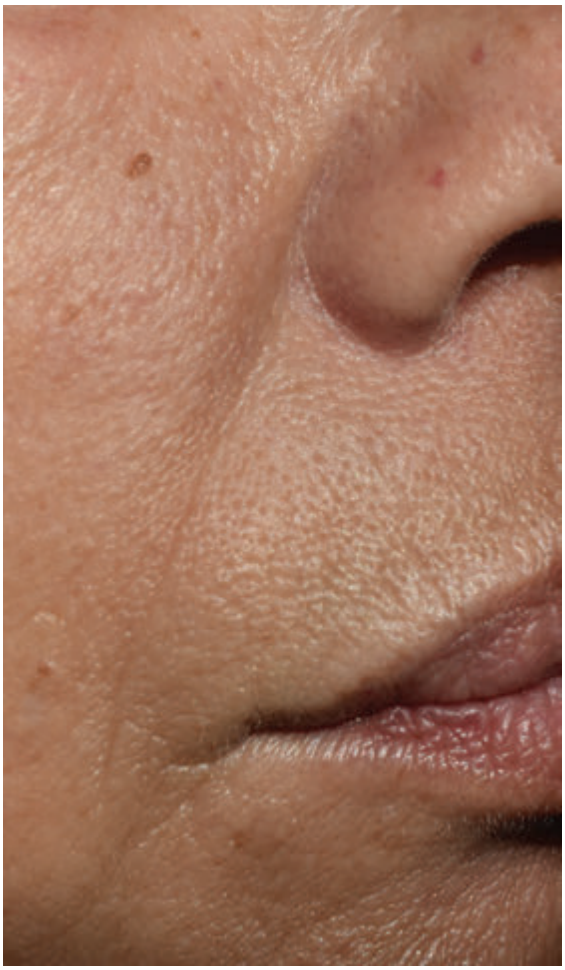


Figure 6 Patient 3, after injection, 1 year.

Discussion

Several methods of isolating SVF have been reported [10], the choice of the enzymatic isolation was motivated by (i) the number of SVF cells obtained is much greater in this case [11], without bursting of mature adipocytes [12] and inducing changes in gene expression in tissue subpopulation [13]; (ii) according to European Medical Agency, enzymatic extraction is not included in the list of substantial modifications that can affected cells.

For the procedure it was required 150 mL of densified fat. This quantity was not reached for 2 patients. BMI is not objectively a good indicator since patient with an important muscle mass have a high BMI, but a low body fat. A simple body impedance analyzer would be better to screen eligible patient. Furthermore, with Adisculpt, shear stress induced by centrifugation is deleterious for cell viability since it breaks matrix as described [14]. So, the Coleman method or decantation for fat densification is recommended.

Our clinical results are in accordance with previous investigation regarding safety and efficiency of SVF injection [15]. No systemic adverse reaction had been observed. All other adverse reactions were due to the lesion induced by the canula.

Regarding inflammation, during AmeaCell® procedure, the extracted SVF cells are stripped of red blood cells, which are known to induce a pro-inflammatory response [16]. The absence of immunoreaction is due to autologous SVF and MatriCS co-injection. Bowles et al. [17] has shown the immunomodulatory effects of SVF by promoting alternative activation of macrophages to repair tissue damage [7]. Adipose tissue displays different physiological functions, having different extracellular matrix compositions and secreted bioactive molecules according to the site of harvesting. The subcutaneous adipose tissue is more indicated for this application compared to the visceral one, because it contains less lymphocytes, neutrophils and macrophages infiltration that promote inflammation [18]. This inflammation has a negative impact on the graft survival even if adipose stem cells display anti-inflammatory action [19]. The co-injection of MatriCS and SVF is a safe process and improved the efficiency in terms of fate of injected cells.

Unfortunately, the method to quantify the evolution of wrinkles volume from Quantificare, did not provide exploitable data. The assessment of the WSRS by the investigators and the GAIS by both investigators and patients gave relevant information. Whatever the WSRS score at baseline, after injection procedure, the score improved in 88% to 100% of the patients. After one year, the WSRS score came to the initial level for half of the patients. This is in accordance with the GAIS scored by both investigators and patients. SVF are far more heterogeneous compared to the homogeneous expanded ADSC. Charles-de-Sà et al. [20] has compared antiaging treatment on patients with SVF enzymatically extracted or with expanded ADSC and concluded that modifications induced are quite similar in both cases. SVF and ADSC are different in cell composition and immunophenotype characteristics. SVF are a mix of several cell types, mainly ADSC (about 30%), endothelial progenitors (about 20%) and immune cells (about 48%) [21] that

can act synergistically to reduce aging effect on skin. It is to note that with expanded ADSC, richness in cell types is lost. Liu et al. [22] has showed that SVF including neutrophils, initiated downstream responses of macrophage infiltration, stimulated vessel formation, and regulated inflammation level, thus had a huge impact on long-term retention rate. Macrophages and their polarization, influence blood-derived stem cell infiltration, indicating that macrophages were crucial for tissue revasculatization [23]. The large variety of cell types in the freshly isolated SVF represents a significant advantage in wound healing [24].

During Ameacell® procedure, the matrix is concentrated and partially decellularized, then pre-conditioned. MatriCs is composed of autologous and concentrated glycosaminoglycans, fibronectin, elastin, collagen, growth factors. It has been shown that hypoxic conditions significantly increased the ability of ADSC to adhere to vascular cell adhesion molecule-1 (VCAM-1) and endothelial intercellular adhesion molecule-1 (ICAM1) present in the ECM [25]. Adhesion is the first step for adherent cells such as ADSC for proliferation then differentiation. Hypoxia has no effect on the phenotype and functionality of ADCS but enhances proliferation and ability of ADSC to secrete VEGF-A, bFGF, contributing to angiogenesis, immunosuppressive capacity, tissue repair and regeneration. It has been demonstrated that hypoxic-treated ADSC display a lower percentage of apoptotic cells compared to normoxic conditions [26]. This innovative matrix pre-conditioning is important to improve cell adhesion and consequently skin regeneration. These preliminary data suggests that MatriCs and SVF cells obtained from AmeaCell® medical device could represent an efficient new product for naturally and a long-lasting term, improving skin aesthetical appearance.

Conclusion

We demonstrate in our study that our technique including supplementation of SVF with a pre-conditioned MatriCS exhibits several advantages including safety, reduction of wrinkles and improvement of skin quality.

Conflict of Interest

NC, CW, AF, JLN had no conflict of interest; BD, VS were employees of Symbioken.

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Ethical Approval

This research has been approved by the Ethic Committee for Clinical trial of Erasme Hospital, Belgium.

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