# Evaluation of human allogeneic collagen gel for correction of nasolabial folds using non-invasive measurement techniques

Mansour Nassiri Kashani, MD [1]
Ali Rajabi Estarabadi, MD [1]
Hamed Zartab, MD, MSc [1]
Parisa Khoshpouri, MD [1]
Pegah Khoshpouri, MD [1]
Hamed Hosseini, MD [2]
Amir Hossein Tavakoli, MD [3]
Alireza Firooz, MD [1,2]

*1. Center for Research & Training in Skin Diseases & Leprosy, Tehran University of Medical Sciences, Tehran, Iran*
*2. Clinical Trial Center, Tehran University of Medical Sciences, Tehran, Iran*
*3. Iranian Tissue Bank and Research Center, Tehran University of Medical Sciences, Tehran, Iran*

*Corresponding Author:*
*Ali Rajabi Estarabadi, MD*
*Address: 415 Taleghani Avenue, Tehran 1416613675, Iran*
*Email: dralirajabi@yahoo.com*

*Conflict of Interest: None to declare*

*Received: 20 April 2015*
*Accepted: 18 June 2015*

**Background:** With aging, the facial folds, grooves, and sagging tissue become more prominent. It is characterized by loss of the collagen mass in the dermis and an increased array of elastin whirls in the deeper dermis. The aim of this study was to determine whether correction of nasolabial folds could be achieved using an allogeneic collagen product.

**Methods:** Nine healthy volunteers participated in this before-after, pilot clinical trial. Human allogeneic collagen (Collagel, Kimia Teb Rahavard Co., Tehran, Iran) was injected in both nasolabial folds of the volunteers. Skin hydration was measured using the Corneometer® 580 device (CK GmbH, Cologne, Germany). Ultrasonic dermal changes were measured using an ultrasonography device (22 MHz, TPM, Germany) before and 24 weeks after the injection. One independent investigator assessed the efficacy using standardized photographs before and 24 weeks after injections. The patients' satisfaction rate was also evaluated.

**Results:** All patients showed improvement in wrinkles and the mean satisfaction rate on a 0-10 VAS was 7.4±0.5. The hydration of the stratum corneum increased from 32.32±13.54 to 52.61±12.55 and the echo-density of the dermis increased from 8.05±3.18 to 9.55±3.36 µm 24 weeks after the injection ($P \leq 0.05$). No treatment-related adverse events were reported.

**Conclusion:** Collagel is an effective filler that can provide a safe and effective correction of the nasolabial folds. This correction lasts for at least 24 weeks on ultrasound evaluations. Further larger blind-randomized controlled clinical trials are required to pave the way for suggesting it as a possible therapeutic option.

**Keywords:** human collagen, dermal fillers, nasolabial fold

Iran J Dermatol 2015; 18: 97-103

## INTRODUCTION

With aging, the facial folds, grooves, and sagging tissue become more prominent. Superficial folds are largely due to damages resulting from sunlight and solar elastosis. It is characterized by loss of the collagen mass in the dermis and an increased array of elastin whirls in the deeper dermis. Grooves appear deeper in the nasolabial and marionette zones with the additional feature of fat atrophy. As a result of the loss of the fat volume, the static suspensory ligaments become more lax and the face takes on the attributes of sagging jowls, sagging malar mounds, and nasolabial folds. Therapies for facial rejuvenation are directed at correcting multiple layers. There are four principals of facial rejuvenation: ensuring adequate skeletal framework and support; tightening and repositioning of the

investing musculofascial aponeurotic system of the face and neck (galea, superficial muscular aponeurotic system, and platysma); replacement of the soft tissue volume loss; and re-draping and removal of the excess skin [1].

In the past decade, there has been a major shift in facial rejuvenation toward less invasive and even nonsurgical procedures with less down time and less pain. Recent surgical and nonsurgical facial rejuvenation techniques focus more on volume restoration for contour correction. In line with this, there was a 4-fold increase in fat injections and a 2.5-fold increase in dermal filler injections in the United States between 1992 and 2002 [2]. Also, according to the American Society of Plastic Surgeons, the use of soft-tissue fillers, including calcium hydroxylapatite, collagen, fat, and hyaluronic acid, for soft-tissue augmentation increased 133% from 2000 to 2007. The increase in the popularity of soft-tissue fillers is understandable, particularly because they are minimally invasive and are effective for restoring the lost volume and correcting contour deficiencies to the aging face [3]. Volume restoration using dermal fillers can rebalance the facial proportion, increase symmetry, and, produce a younger appearance by reducing the wrinkles [2]. Although soft tissue augmentation dates back to more than 100 years ago, the search for ideal filler still continues. A variety of filling agents have been tried with varying success rates. Due to the fact that aging is a continuous process, temporary fillers should be preferred over permanent ones [4]. Products marketed for soft-tissue augmentation include those derived from bovine and human collagen, calcium hydroxyapatite, poly(methyl methacrylate), poly-L-lactic acid, avian-derived hyaluronic acid, and non–animal-stabilized hyaluronic acid (NASHA) [3].

Dermal fillers are generally divided into two categories: absorbable and non-absorbable. Absorbable fillers are divided into synthetic and natural. Absorbable synthetic fillers consist of hydroxyapatite calcium microspheres. Absorbable natural fillers contain bovine collagen, human collagen, porcine collagen and hyaluronic acid. Non-absorbable fillers include silicone and poly methyl methacrylate microsphere [1]. A drawback of the fillers is that their duration of effect is limited: a few months for autologous fat transfer and collagen (human or bovine derived), and approximately 12 months for hyaluronic acid preparations and calcium hydroxylapatite. Therefore, it is desirable to identify fillers for soft-tissue augmentation that have a duration of effect that extends beyond what is currently available [3,5]. The ideal dermal filler is one that is biocompatible, predictable, adjustable to the anatomy of the patient, long-lasting, reversible, and natural in appearance [1,6]. The use of autologous human fibroblast injections is expected to result in a longer duration of correction based on local production of collagen as well as decreased collagen degradation [7].

Bovine collagen was the first filler available and included the formulations of Zyderm® and Zyplast® (Allergan, Irvine, CA, USA). These products ranged from 35 to 65 mg/mL bovine collagen and had 0.3% lidocaine. The cross-linked "plast" product was made with glutaraldehyde. A skin test was required to screen for the 1.5% to 3% incidence of delayed type hypersensitivity [1]. Dermal correction with bovine collagen implants is generally of short duration requiring frequent re-injections, and hypersensitivity reactions are relatively common [5]. Human collagen equivalents in dosing and cross-linking (Cosmoderm® and Cosmoplast®; Allergan) were introduced to eliminate the need for allergy testing. All collagen products have clinical effects lasting from 1 to 4 months. The main clinical advantage of the human collagen products is their ability to correct the most superficial lines with smooth flow characteristics as their carrier is phosphate-buffered saline. The duration of clinical effects has not reached that of the hyaluronic acid gels as demonstrated in a blinded comparative study against Zyplast [1]. Human collagen preparations have efficacy and safety profiles similar to those of bovine collagen preparations [8].

Iranian Tissue Product (ITP) Collagen (Collagel) is a new human allogeneic collagen product which is available in gel formation. The purpose of this study was to determine the safety, efficacy and persistence of this allogeneic human collagen product in correction of nasolabial folds.

## PARTICIPANTS AND METHODS

### Participants

Nine healthy volunteers (5 females, 4 males

- age range: 26-46 years) with mild to moderate nasolabial folds were recruited for this study. The exclusion criteria were a recent history of any skin diseases or operation in the previous 3 months, any systemic disease that could affect the skin status, pregnancy, any other previous cosmetic interventions on the nasolabial folds such as filler injection, laser therapy, peeling, or nonablative rejuvenation procedures in the year prior to the start day of the study, and a history of smoking. This study was approved by the Review Board and Ethics Committee of Iranian Tissues Bank Preparation and Research Center, Tehran University of Medical Sciences, Tehran, Iran. All the participants provided the investigators with written informed consents for the intervention and for clinical photography.

### Clinical assessment

Participants were instructed not to use any pharmaceutical, cosmeceutical or hygienic products on their skin from the night prior to the first session (in which the injection was given). On the first day, prior to the treatment initiation, participants were asked to rest and relax for 20 minutes in an standard atmosphere (20-22°C, 30-60% humidity) and then the hydration of the epidermal stratum corneum of the nasolabial folds was measured and recorded using the Corneometer® 580 (CK GmbH, Cologne, Germany). Then, skin ultrasonography (22 MHz, TPM, Germany) was done on treatment areas before and immediately after the injection for evaluating the density of the injected area. Hydration measurement was repeated 2, 12, and 24 weeks after the injection and sonographic assessment was done again on the final visit (24 weeks after the injection) to assess the stability of the injections.

Digital photography (Nikon® camera, Japan) of the front face was performed at baseline (as mentioned above) and immediately after the injection, and 24 weeks from the time of treatment initiation. The Investigator Global Assessment (IGA) was recorded by a blind dermatologist after 24 weeks, using a five-point scale (compared with the baseline photographs) using the following grading scale:

Worse (-1): exacerbation,

No change (0): improvement of 24% or less,

Fair (1): improvement of 25-49%,

Good (2): improvement of 50- 74,

Excellent (3): improvement of 75% or more [9].

The subject's satisfaction with the treatment was also assessed after 24 weeks using a 10-score verbal assessment scale (VAS), with the score 0 indicating no satisfaction at all and the score 10 indicating an ideal result. Any possible adverse event was asked and recorded throughout the trial duration.

### Intervention

The participants received one single treatment in the first session for their right and left nasolabial folds using 2-3 milliliters of ITP Collagel made by Iranian Tissue Product Co, Tehran, Iran, with the standard technique for the injection of dermal fillers.

To anaesthetize the region, a thin layer of EMLA cream (a local anesthetic containing lidocaine and prilocaine) was applied to both nasolabial folds which remained in place for 30 minutes. ITP Collagel was injected with 23G needles into the nasolabial folds of each participant at the mid-dermal level. A linear threading injection technique was used.

### Statistical analysis

The mean value and standard deviation were used to describe quantitative variables and discrete variables were described by using frequency and percentage frequency. Linear models were used in order to evaluate the changes of dermal density and hydration over time, considering the data correlation between repeated measurements over time and correlation between measurements on each side of the face of the cases. The level of significance was set at 0.05.

## RESULTS

Eighteen sites (right and left side nasolabial folds of 5 females and 4 males) were evaluated. All the participants completed the treatment period and no deviation from the protocol was observed. The mean age of the participants was 38.4±7.6 years and the mean dermal density and hydration before treatment were 8.1±3.2 and 32.3±13.5, respectively (Table 1).

**Table 1.** Characteristics of patients before treatment

| Participants | Gender | Age | Epidermal Hydration* | | Dermal density** | |
|---|---|---|---|---|---|---|
| | | | Right | Left | Right | Left |
| 1 | F | 44 | 21.30 | 19.73 | 10 | 14 |
| 2 | M | 33 | 12.91 | 28.50 | 6 | 7 |
| 3 | M | 41 | 48.28 | 35.53 | 3 | 5 |
| 4 | F | 46 | 38.30 | 42.80 | 11 | 5 |
| 5 | F | 44 | 43.90 | 33.90 | 5 | 10 |
| 6 | F | 30 | 17.97 | 20.57 | 11 | 12 |
| 7 | F | 43 | 13.83 | 17.33 | 9 | 12 |
| 8 | M | 26 | 56.30 | 40.37 | 8 | 6 |
| 9 | M | 41 | 41.97 | 48.33 | 4 | 7 |

*Epidermal hydration of treatment area using Corneometer®
**Dermal density of treatment area using 22 MHz ultrasonic probe

**Table 2.** Changes in epidermal hydration over time.

| | Before* | 2 weeks | 12 weeks | 24 weeks | *P* |
|---|---|---|---|---|---|
| Epidermal hydration | 32.32±13.54 | 43.53±8.84 | 48.19±10.61 | 52.61±12.55 | 0.001 |

There was a significant increasing trend in hydration over time (Table 2, Figure 1, *P* =0.001). Changes of the dermal density, measured by ultrasonography, immediately and 24 weeks after the injection showed an increasing trend; however, the changes were not statistically significant (*P*=0.180, Figures 2 and 3). The mean satisfaction score of the participants at the end of 24 weeks after the injection was 7.4±0.5. Furthermore, the mean score given by a blinded dermatologist using the standard pictures taken during the course of study was 1.3±0.2 (Figure 4). No side effects were observed due to the intervention.

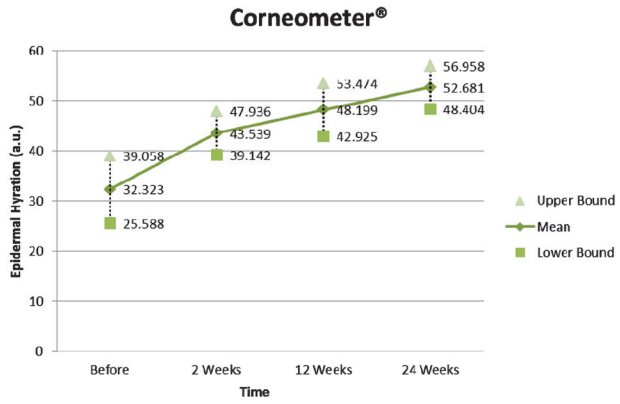


**Figure 1.** Increasing in epidermal hydration 2, 12 and 24 weeks after injection of Collagel in nasolabial folds by using Corneometer® 580 device. (a.u: arbitrary unit).

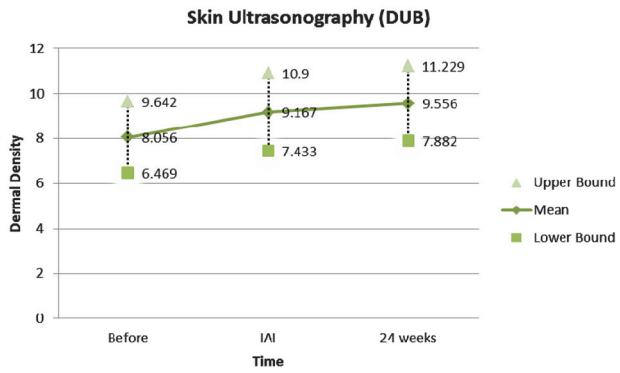


**Figure 2.** Changes of dermal density 24 weeks after injection of Collagel in nasolabial folds, measured by ultrasonography. (IAI: Immediately after injection)

## DISCUSSION

Ideal filling materials for improving skin wrinkles should have properties such as good biocompatibility, stability, and few side effects, and should be easy to use. Several attempts were made to develop such a product in the past decades. One of these products is human collagen [10-12].

The use of human collagen for improving skin wrinkles reflects interesting natural effects that make it a good alternative for other substances. Clinical studies and histological analysis have shown a sustained increase in the dermal thickness and collagen formation which was confirmed in our ultrasound assessments [13,14].

The advantages of allogeneic collagen grafts are biocompatibility and no need for hypersensitivity reaction tests before use, safety and traceability of all human sources for a long period of time, screening all donors for serological tests such as ELISA and PCR assay with microbiological culture tests and assurance of tissue safety before processing the product, good volume enhancement, helping

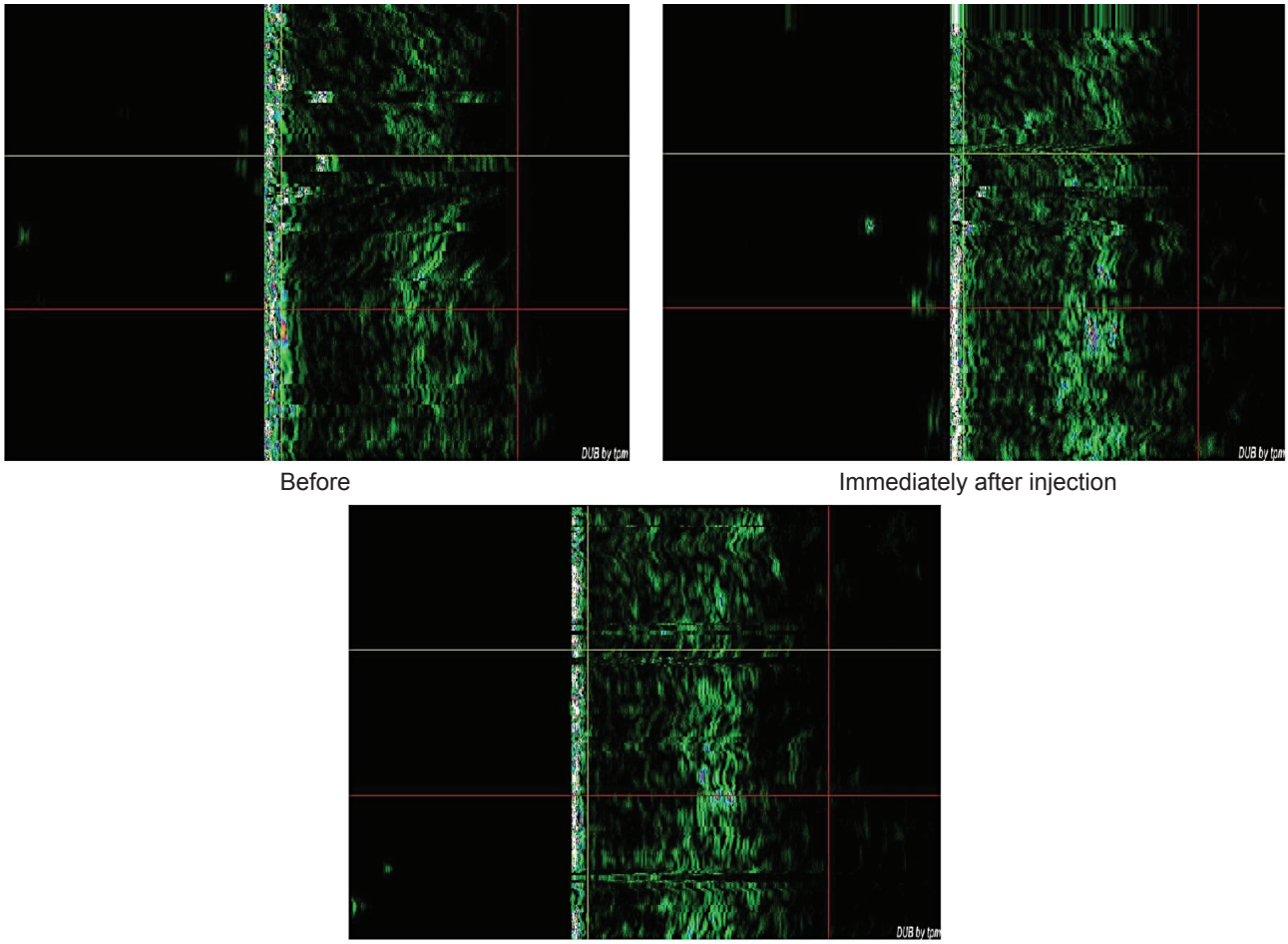


**Figure 3.** Sonographic views of nasolabial folds before, immediately and 24 weeks after injection of collagen by using 22 MHz ultrasonography probe.

with the maintenance of normal tissue aesthetic reconstruction, and their ability to act as a soft tissue augmenting agent and volume enhancer in membranous form. It fills the space evenly and is malleable yet remains in place, giving reliable early natural results. However, there are limited sources for allogeneic tissue preparation and it is very expensive worldwide, which are some of its drawbacks for users.

Narins *et al.* showed that the xenogeneic (porcine) collagen was effective and persistent (as evaluated for up to 6 months) as a hyaluronic acid-based dermal filler and the current gold standard for treatment [15]. In another study, after a long-term follow-up of the patients, they showed that the clinical effects persisted for up to 9 months in 95.3% of the patients and for up to 12 months in 76.5% of the patients [5]. Also, similar to our study, no side effects were reported during the studies.

Collagel as an autologous human collagen filler for soft tissue correction is an attractive prospect. The findings of this study showed a clinical improvement in nasolabial folds of the participants as well as an improvement in the density of the dermal collagen and epidermal hydration, which can both contribute to this effect. Therefore, larger blinded, randomized controlled comparative clinical trials are required to demonstrate how much application of the human collagen is effective in comparison with other dermal fillers.

## Acknowledgments

This research was funded by a research grant provided by Kimia Teb Rahavard Co.

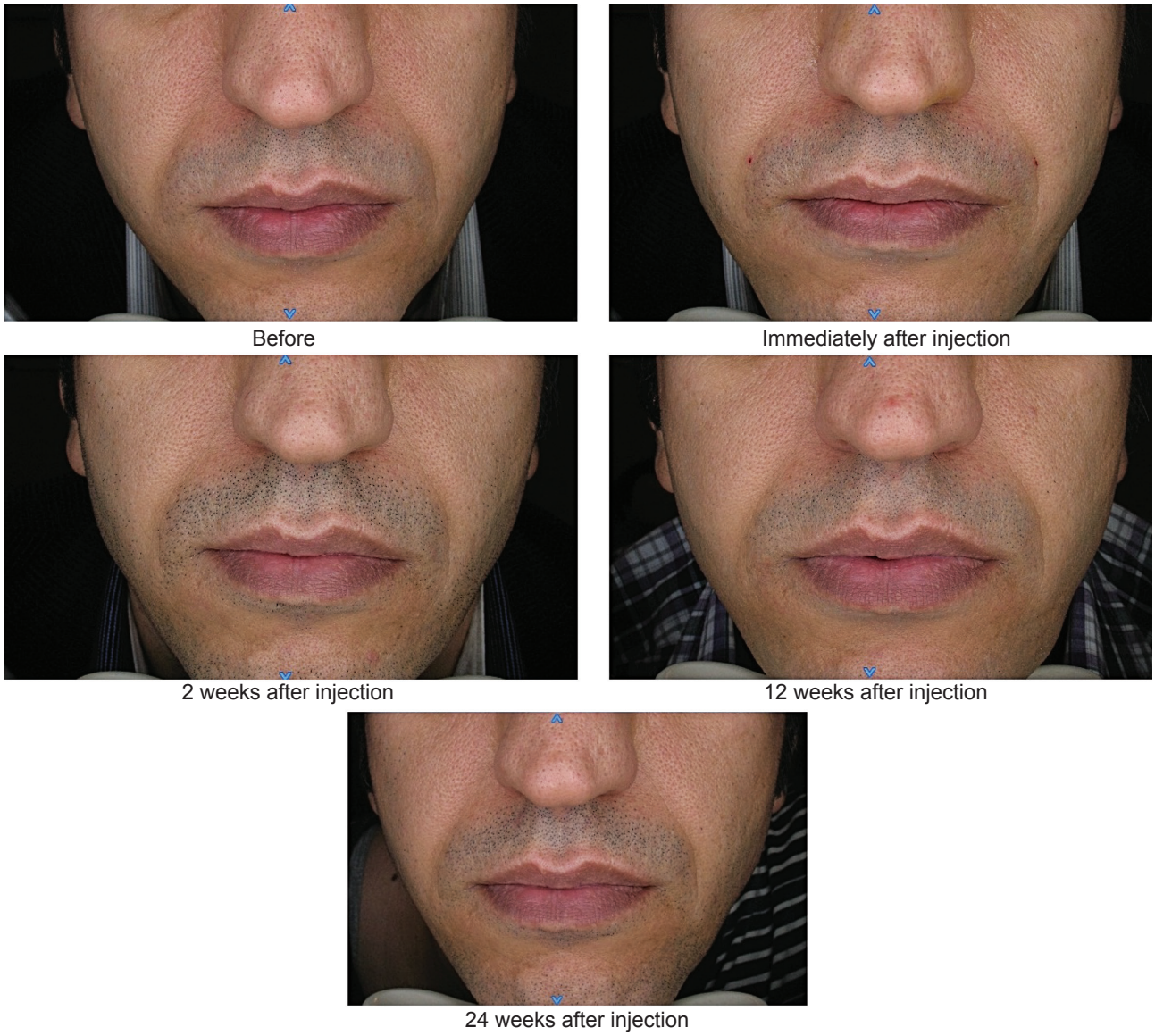


**Figure 4.** A 41-year-old male patient, before, immediately, 2, 12 and 24 weeks after injection of collagen in nasolabial folds.

## REFERENCES


1. Newman J. Review of soft tissue augmentation in the face. Clin Cosmet Investig Dermatol 2009;2:141-50.
2. Monstrey SJ, Pitaru S, Hamdi M, et al. A two-stage phase I trial of Evolence30 collagen for soft-tissue contour correction. Plas Reconstr Surg 2007;303-11.
3. Narins RS, Baumann L, Brandt FS, et al. A randomized study of the efficacy and safety of injectable poly L-lactic acid versus human-based collagen implant in the treatment of nasolabial fold wrinkles. J Am Acad Dermatol 2010;62:448-62.
4. Sales AG, Lotierzo PH, Gimenez R, et al. Evaluation of poly-L-lactic acid implant for treatment of the nasolabial fold: 3-year follow-up evaluation. Aesth Plast Surg 2008;32:753-6.
5. Narins RS, Brandt FS, Lorenc ZP, et al. Twelve-month persistency of a novel ribose-cross-linked collagen dermal filler. Dermatol Surg 2008;34:S31-9.
6. Bauman LS, Shamban AT, Lupo MP, et al. Comparison of smooth-gel hyaluronic acid dermal fillers with cross-linked bovine collagen: a multicenter, double-masked, randomized, within-subject study. Dermatol Surg 2007;33:S128-35.
7. West TB, Alster TS. Autologous human collagen and dermal fibroblasts for soft tissue augmentation. Dermatol Surg 1998;24:510-12.
8. Weinkle S. Efficacy and tolerability of admixing 0.3% lidocaine with Dermicol P-35 27G for the treatment of nasolabial fold. Dermatol Surg 2010;36:316-20.
9. Choi YJ, Lee JY, Ahn JY, et al. The safety and efficacy of a combined diode laser and bipolar radiofrequency compared with combined infrared light and bipolar radiofrequency for skin rejuvenation. Indian J Dermatol

Venereol Leprol 2012;78:146-52.

10. Boss WK,Usal H, Chernoff G, et al. Autologous cultured fibroblasts as cellulartherapy in plastic surgery. Clin Plast Surg 2000;27:613–26.
11. Boss WK, Usal H, Fodor PB,Chernoff G. Autologouscultured fibroblasts: A protein repair system. Ann Plast Surg 2000;44:536–42.
12. Keller G, Sebastian J, Lacombe U, et al. Safety of injectable autologous human fibroblasts. Bull Exp Biol Med 2000;130:786–9.
13. Watson D, Keller GS, Lacombe V, et al. Autologous fibroblasts for treatmentof facial rhytids and dermal depressions: A pilot study.Arch. Facial Plast Surg 1999;1:165–70.
14. Weiss RA, Weiss MA, Beasley KL, Munavalli G. Autologous cultured fibroblast injection for facial contour deformities: A prospective, placebo-controlled, phase III clinical trial. Dermatol Surg 2007;33:263–8.
15. Narins RS, Brandt FS, Lorenc ZP, et al. A randomized, multicenter study of the safety and efficacy of dermicol-p35 and non-animal-stabilized hyaluronic acid gel for the correction of nasolabial folds. Dermatol Surg 2007;33 Suppl 2:S213-21.