International Journal of Dermatology

Original Article

Dermatologic Surgery and Cosmetic Dermatology

Comparison of microneedling and CO_2 laser with adipose-derived stem cells for facial rejuvenation: a randomized split-face study

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Keywords
adipose-derived stem cells; facial rejuvenation; microneedling; split-face; carbon dioxide fractional resurfacing; ablative laser.

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Conflict of interest: None.

Funding source: None.

doi: 10.1111/ijd.17554

Abstract

Background Facial aging, characterized by structural decline and loss of collagen and elastin, has led to increased demand for rejuvenation treatments. Adipose-derived stem cells (ADSCs) have emerged as a promising option, but comparative studies on their application methods are limited.

Objective Our aim was to compare the efficacy of ADSC combined with microneedling or CO_2 laser for facial rejuvenation.

Methods Twenty-seven participants were randomized into two groups: Microneedling (MN, $n = 14$) or CO_2 laser ($n = 13$). Each group underwent three treatment sessions at 4-week intervals. The ADSC solution was applied to one side and the placebo to the other using a split-face design. We performed objective evaluations (UV spots, brown spots, wrinkles, texture, pores, red areas, and porphyrins) and subjective assessments, including clinical photographs, patient satisfaction scales, and histological analysis of skin biopsies.

Results The CO_2 laser with the ADSC group showed significantly more significant improvements in UV spots ($P = 0.002$) and wrinkles ($P = 0.002$) compared to the MN with the ADSC group. Histological analysis revealed superior elastin fibers and epidermal thickness improvements with CO_2 laser treatment. Patient satisfaction was higher in the CO_2 laser group, with 84.6% reporting complete satisfaction compared to 50% in the MN group.

Conclusions The combination of CO_2 laser with ADSCs demonstrated superior efficacy for facial rejuvenation compared to MN with ADSCs. This approach improved UV spots, wrinkles, skin structure, and overall patient satisfaction. Further studies with larger cohorts and extended follow-up are needed to confirm long-term efficacy.

Introduction

Facial rejuvenation has become an increasingly popular area of aesthetic medicine, with patients seeking effective, minimally invasive treatments to address signs of aging. Among the various approaches, microneedling (MN) and carbon dioxide (CO_2) laser therapy have emerged as prominent skin rejuvenation techniques, each with unique mechanisms of action and efficacy profiles. Concurrently, the field of regenerative medicine has introduced adipose-derived stem cells (ADSCs) as a promising adjunct to these treatments, potentially enhancing their regenerative capabilities.[1,2]

Microneedling, a collagen induction therapy, involves creating controlled micro-injuries to the skin, stimulating the natural wound healing process, and promoting collagen and elastin production.[3] On the other hand, fractional CO_2 laser therapy utilizes thermal energy to create microscopic treatment zones, triggering skin remodeling and collagen synthesis.[4] While both techniques have demonstrated efficacy in improving skin texture, reducing fine lines, and addressing photoaging, their comparative effectiveness when combined with ADSCs remains understudied.[5–7]

This study aims to address this knowledge gap by directly comparing the efficacy of MN and CO_2 laser therapy when combined with ADSCs for facial rejuvenation. We hypothesized that combining ADSCs with either MN or CO_2 laser therapy would yield superior results compared to these treatments alone and that one combination might prove more effective than the other. To test this hypothesis, we conducted a randomized, split-face, double-blind study evaluating objective and subjective measures of skin improvement, as well as histological changes, in participants undergoing these combined treatments.

Materials and methods

This 12-week randomized split-face comparative study evaluated the clinical efficacy of combining ADSC with MN and CO_2 laser treatments for facial skin aging. It adhered to the Declaration of Helsinki principles and received Institutional Review Board approval. All participants provided informed consent before enrollment, during which we also explained the study.

Participants were randomly divided into two groups based on the treatment received: MN and CO_2 laser (Figure 1). Each group underwent three intervention sessions 4 weeks apart, with follow-up 4 weeks post-final intervention. A person external to the study oversaw the randomization process. The complete process for creating the two comparison groups was performed using the Microsoft Excel RAND function. The individual overseeing the randomization process concealed the allocation sequence until each participant was registered in the trial. Each session involved applying ADSC to one side of the face and a saline solution as a control on the other, with the intervention also assigned randomly.

We included male and female participants aged 45–65, classified as classes II–IV on the Glogau photoaging scale. We excluded participants who were pregnant, active smokers, had an acute inflammatory or infectious facial condition or a history of keloid scarring, and concurrently used other skin treatments. Participants were also advised not to use any other skin products during the trial.

After liposuction, adipose tissue was transported in a physiological saline solution (NaCl 0.9%) to the laboratory, where it was washed three times to remove blood and debris. The tissue was digested for 45 minutes with agitation using Collagenase NB 6 GMP Grade at 0.1% concentration (Nordmark Pharma GmbH, Uetersen, Germany). Following digestion, the sample was centrifuged at 1500 rotations per minute (rpm) for 10 minutes to obtain a cell concentrate, which was then resuspended and cultured in DMEM/Ham's F-12 medium (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% NuSera Serum Replacement (HiMedia Laboratories LLC, Kelton, PA, USA) and 1% Anti-Anti (Gibco, Thermo Fisher Scientific). Cultures were maintained at 5% CO_2 at 37°C with 80% relative humidity.

Upon reaching 80% confluence, cells were enzymatically dissociated using 2.5% trypsin (Gibco, Thermo Fisher Scientific), washed with 1× PBS (Gibco, Thermo Fisher Scientific), and centrifuged at 1500 rpm for 10 minutes. The ADSCs were resuspended in physiological saline for patient application, with each dose consisting of 1×10^6 mesenchymal stem cells (MSCs).

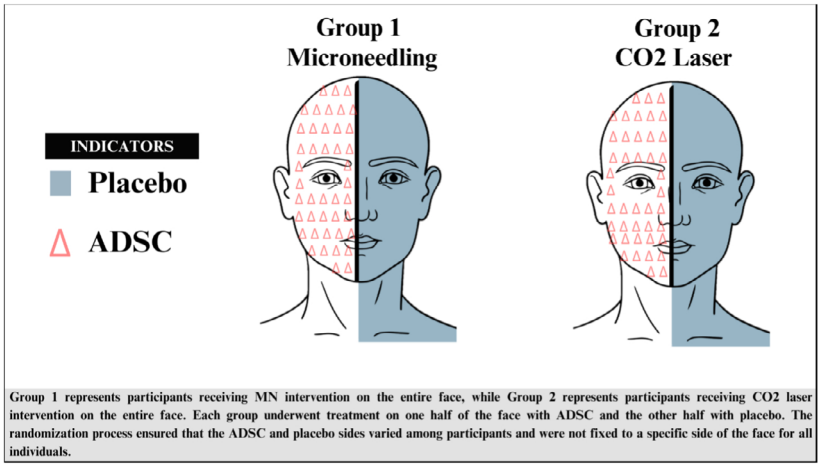

Figure 1 Graphical representation of the different intervention modalities and ADSC implementation

The adherent cells displaying MSC morphology were successfully characterized based on the detection of specific stem cell markers, as per the International Society for Cellular Therapy guidelines. Immunophenotyping was performed using flow cytometry, with negative isotype controls for each antibody and unstained controls to exclude nonspecific signals. The analysis confirmed the expression of mesenchymal markers CD105, CD73, and CD44, while hematopoietic markers CD45 and CD14 were absent, consistent with the expected MSC phenotype (data not shown). These results indicate successful isolation and characterization of MSCs.

ADSC characterization was performed by flow cytometry, assessing positive markers CD73, CD90, and CD105, as well as negative markers CD45 and CD34. For immunophenotyping, approximately 3×10^6 cells at passage three were detached using 2.5% trypsin (Gibco, Thermo Fisher Scientific), neutralized with fetal bovine serum (Gibco, Thermo Fisher Scientific), and pelleted. Cells were resuspended in staining buffer, and 10 μL of each antibody was added at a 1:10 dilution (Table A1). After a 20-minute incubation in the dark, samples were washed, centrifuged at 2000 rpm for 10 minutes at 4°C, and analyzed by flow cytometry using FlowJo software v10.10 (Tree Star Inc; Ashland, OR). Isotype controls were used to eliminate nonspecific signals.

For the objective evaluation in this study, we used the VISIA® system (Canfield, USA) to numerically measure multiple skin parameters, including spots, UV spots, brown spots, wrinkles, texture, pores, red areas, and porphyrins. For the subjective evaluation, at each visit, with prior consent, participants were photographed under consistent conditions using a Canon® PowerShot SX530 HS camera (Tokyo, Japan). Two certified dermatologists assessed these photographs to rate the physician global assessment (PGA) based on improvement, ranging from 0 (*worsened*) to 5 (*excellent improvement*). This was assessed in a double-blind manner, and the evaluators were unaware of which side received the placebo and which side received the ADSC solution.

We used the patient subjective satisfaction scale (PS) for participants to rate their satisfaction on each side of their face on a 0–4 scale, with 0 being not satisfied at all and 4 being completely satisfied. We asked two questions in a double-blind manner, meaning neither the participant nor the investigator knew which side of the face was treated with a placebo and which side had ADSC. The questions were: (1) How satisfied are you with the appearance of the right side of your face? and (2) How satisfied are you with the appearance of the left side of your face? These questions were asked before the study began and 1 month after completion. Adverse events such as pain, edema, erythema, and scars during each visit were also self-reported by participants.

We prepped each participant by washing their faces with mild soap and applying a local anesthetic cream (tetracaine 7%, lidocaine 23%) 45 minutes before the intervention. In Group 1, MN was conducted across the face to a depth of 2.5 mm using Dr. Pen® A6 (Perth, Western Australia), followed by applying 3 ml ADSC solution on one half of the face and 3 ml saline solution on the other half. Group 2 underwent fractional CO_2 laser (Jeisys®, South Korea) intervention on the entire face, followed by applying 3 ml ADSC solution and 3 ml saline solution to respective halves of the face.

We also collected skin tissue samples from both sides of participants' faces using a 4 mm punch biopsy at the initial visit and 1 month post-intervention. An experienced, independent dermatopathologist processed the samples using multiple staining techniques, including hematoxylin and eosin, Masson's trichrome, and Verhoeff-van Gieson. It was assessed for collagen bundles, elastin fibers, epidermal thickness, and solar elastosis. These parameters were evaluated using a subjective graded scale from 0 (*none*) to 3 (*marked*). This detailed histological analysis provided a comprehensive assessment of the structural changes in the skin following the intervention in both groups.

Statistical analysis

We employed a "split-face" study modality in the two groups, where each face was divided into one half treated with ADSC and the other half treated with a placebo solution, post-intervention with MN or CO_2 laser. The evaluated variables included the objective measurement of spots, UV spots, brown spots, red areas, wrinkles, texture, pores, and porphyrins.

Initially, we conducted a descriptive analysis of the studied variables, providing measures of central tendency and dispersion for continuous variables and frequencies and percentages for categorical variables. We used the chi-squared and Mann–Whitney tests to detect baseline age and sex distribution differences between the CO_2 laser and MN intervention groups. As a result of the number of patients and the wide variation in the standard deviations of each continuous objective variable, we opted for a nonparametric analysis. For our primary objective, we used the Mann–Whitney *U* test to compare changes between the CO_2 and MN groups since these are independent groups. For our secondary objectives, we used paired Wilcoxon tests to compare the difference between the participants' start and end of the ADSC and placebo-treated sides. This analysis was carried out separately for the groups subjected to MN and CO_2 laser.

All statistical analysis was performed using SPSS software version 29. Tables and figures illustrated the distribution and changes in the evaluated variables, as well as the comparison of treatment effects between groups and application methods.

Results

We included 27 participants aged 46–65 years. Group 1 (MN) comprised 14 participants, while Group 2 (CO_2 laser) had 13 participants. All participants completed the study. In the MN group, there were 12 female participants (85.71%), with ages

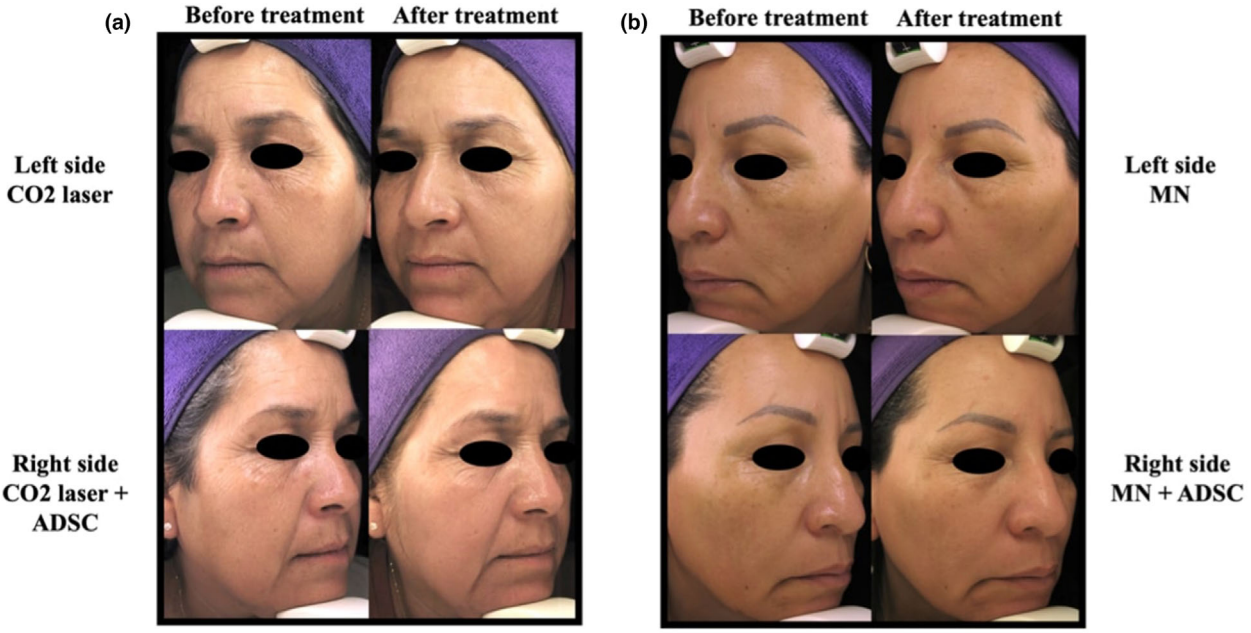

Figure 2 Clinical photographs before and after treatment in two patients who underwent CO_2 laser (a) and microneedling (b)

Table 1 Median changes in objective evaluations for saline versus ADSC with microneedling and CO_2 laser

	Microneedling		CO_2	
Variable	**ADSC**	**Saline**	**ADSC**	**Saline**
Spots	−3 (−13.75 to −1)	1.50 (−13 to 7.2)	−21 (−27 to 8)	−23 (−32 to 5.5)
UV spots[a]	−32 (−91 to 10.5)	−11.00 (−87 to 7.2)	−108 (−148 to −40)	−126 (−204 to 14.5)
Brown spots	−13 (−32.7 to 0.75)	−2.50 (−31.25 to 8.25)	4 (−30 to 57)	4 (−84.5 to 17)
Wrinkles[a]	1 (−1.50 to 2)	0.50 (−4.25 to 6)	−3 (−6 to −1.5)	−5 (−12 to 1)
Texture	−21 (−123 to 125)	−31.50 (−101.75 to 127.75)	−103 (−259 to −9)	−168 (−260.5 to 167.5)
Pores[b]	−21 (−145.7 to 39)	−23.00 (−71.75 to 60.25)	−73 (−117 to 26)	−97 (−133 to 25)
Erythematous áreas[c]	14 (−27.7 to 36)	−16.50 (−43.25 to 9.75)	3 (−33 to 41.5)	−30 (−66 to −0.5)
Porphyrins	−67 (−104 to 6.7)	−72.00 (−140.75 to 2.25)	−25 (−69 to 2.5)	−18 (−71.5 to 55)

Change represents the difference in medians between the placebo-treated side and ADSC-treated side. Variables are reported as medians (IQR).
ADSC, adipose-derived stem cells; UV, ultraviolet.
[a]Statistically significant *P*-value between ADSC in microneedling and CO_2.
[b]Statistically significant *P*-value between ADSC and saline solution in microneedling group.
[c]Statistically significant *P*-value between ADSC and saline solution in CO_2 group.

ranging from 49 to 60 years and a mean age of 53 years (SD ± 3.7). In the CO_2 laser group, there were 12 female participants (92.31%), with ages ranging from 46 to 65 years and a mean age of 54 years (SD ± 5.79). No statistically significant baseline differences were found between the groups regarding age ($P = 0.064$) or sex ($P = 0.76$).

Objective evaluation

The objective evaluation results were compared for sides treated with ADSC in both groups: MN and CO_2 laser (Figure 2). Table 1 shows the median changes in objective evaluation for saline versus ADSC with MN and CO_2 laser.

Spots and wrinkles

Both interventions showed reduced spots and wrinkles, with the CO_2 laser combined with ADSCs yielding superior results. The objective evaluation indicated reductions in spots and UV spots for the CO_2 laser group, with a median of −21 (IQR: −27 to 8) in spots and −108 (IQR: −148 to −40) in UV spots, compared to the MN group's reductions of −3 (IQR: −13.75 to −1)

Table 2 Physician's global assessment for saline versus ADSC in the microneedling and CO_2 laser groups

	Microneedling				CO_2			
	Saline		ADSC		Saline		ADSC	
Improvement	Evaluator 1, *n* (%)	Evaluator 2, *n* (%)	Evaluator 1, *n* (%)	Evaluator 2, *n* (%)	Evaluator 1, *n* (%)	Evaluator 2, *n* (%)	Evaluator 1, *n* (%)	Evaluator 2, *n* (%)
None (0%)	1 (7)	1 (7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Minimal (0%–25%)	7 (50)	9 (64)	9 (64)	8 (57)	4 (31)	5 (39)	2 (15)	2 (15)
Regular (26%–50%)	5 (36)	3 (21)	4 (29)	5 (36)	3 (23)	2 (15)	5 (39)	5 (39)
Good (51%–75%)	1 (7)	1 (7)	1 (7)	1 (7)	6 (46)	6 (46)	4 (31)	3 (23)
Excellent (76%–100%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (15)	3 (23)

ADSC, adipose-derived stem cell.

in spots and −32 (IQR: −91 to 10.5) in UV spots. The changes in UV spots between groups were statistically significant ($P = 0.002$). Wrinkle reduction was also more pronounced in the CO_2 laser group, with a median of −3 (IQR: −6 to −1.5) compared to a slight increase in the MN group (1, IQR: −1.50 to 2), this difference was also statistically significant ($P = 0.002$).

Texture and pores

Both groups exhibited improvements in skin texture and pore size. The CO_2 laser group showed more significant improvement in texture (median decrease of 103, IQR: −259 to −9) and pores (median decrease of 73, IQR: −117 to 26) compared to the MN group (texture: median decrease of 21, IQR: −123 to 125; pores: median decrease of 21, IQR: −145.7 to 39). However, these differences were not statistically significant.

Erythematous areas and porphyrins

Increases in erythematous areas were observed in both groups, with no significant statistical difference ($P = 0.905$). Porphyrin levels decreased in both groups, with the MN group showing a median of −67 (IQR: −104 to 6.7) and the CO_2 laser group of −25 (IQR: −69 to 2.5) ($P = 0.325$).

Subjective evaluation

Physician global assessment

The PGA results comparing the ADSC-treated sides versus saline for both groups are summarized in Table 2.

The MN-ADSC group had a higher proportion of participants in the minimal improvement category than the CO_2-ADSC group. Conversely, the CO_2-ADSC group had more participants experiencing regular, good, and excellent improvements, indicating a broader range of positive outcomes. Specifically, while evaluators reported most of the participants in the minimal to regular improvement categories, the CO_2-ADSC group had more participants in the good and excellent improvement categories.

Patient satisfaction

The patient satisfaction results comparing the ADSC-treated sides versus saline for MN and CO_2 laser groups are summarized in Table 3. Initially, in the MN group, satisfaction was low: 7.1% of participants reported no satisfaction, 57.1% were slightly satisfied, and 35.7% were moderately satisfied. None of the participants felt very or completely satisfied. By the end of the study, satisfaction improved. No participants reported no or slight satisfaction, 7.1% were moderately satisfied, 42.9% were very satisfied, and 50% were completely satisfied.

In the CO_2 laser group, initial satisfaction was similar, with 23.1% of participants reporting no satisfaction, 30.8% slightly satisfied, and 46.2% moderately satisfied. None of the participants felt very or completely satisfied at the start. However, by the study's end, satisfaction levels had improved dramatically. No participants reported no or slight satisfaction, 15.4% were very satisfied, and 84.6% were completely satisfied.

Comparing the final satisfaction levels between the two groups, the CO_2-ADSC laser group had a higher proportion of participants reporting complete satisfaction (84.6%) compared to the MN-ADSC group (50%). Conversely, the MN-ADSC group had a higher percentage of participants who were very satisfied (42.9%) compared to the CO_2-ADSC laser group (15.4%). These results highlight differences in the effectiveness of the treatments, with the CO_2-ADSC group showing a higher overall rate of complete satisfaction, while the MN-ADSC group

Table 3 Patient subjective satisfaction scale for saline versus ADSC in the microneedling and CO_2 laser groups

	Microneedling				CO_2			
	Saline		ADSC		Saline		ADSC	
Improvement	Initial, *n* (%)	Final, *n* (%)	Initial, *n* (%)	Final, *n* (%)	Initial, *n* (%)	Final, *n* (%)	Initial, *n* (%)	Final, *n* (%)
None	1 (7)	0 (0)	1 (7)	0 (0)	3 (23)	0 (0)	3 (23)	0 (0)
Slightly satisfied	8 (57)	1 (7)	8 (57)	0 (0)	4 (31)	0 (0)	4 (31)	0 (0)
Moderately satisfied	5 (36)	0 (0)	5 (36)	1 (7)	6 (46)	0 (0)	6 (46)	0 (0)
Very satisfied	0 (0)	7 (50)	0 (0)	6 (43)	0 (0)	3 (23)	0 (0)	2 (15)
Completely satisfied	0 (0)	6 (43)	0 (0)	7 (50)	0 (0)	10 (77)	0 (0)	11 (85)

Results obtained at the beginning and end of the study.
ADSC, adipose-derived stem cell.

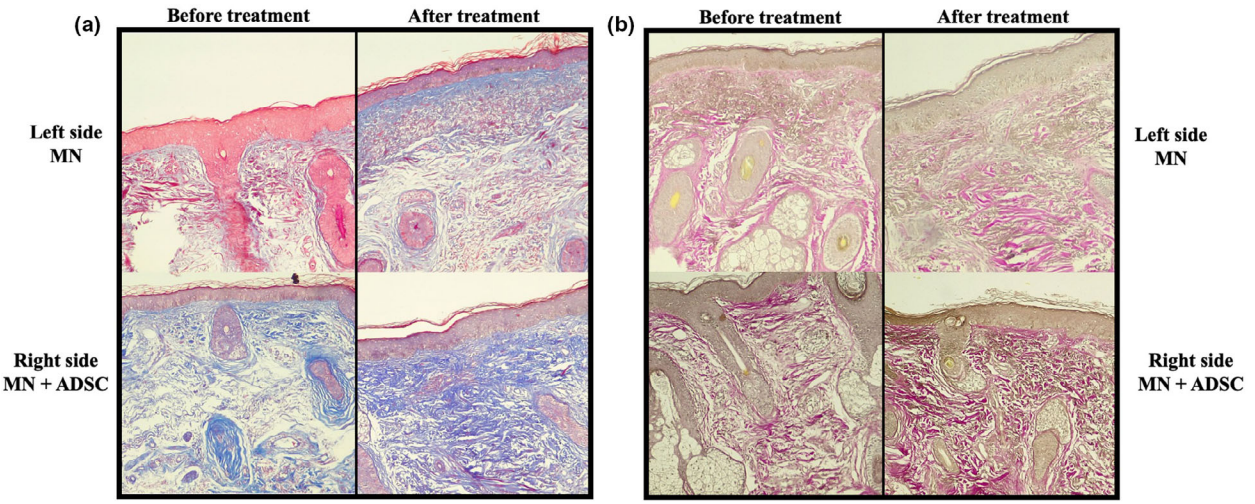

Figure 3 Before and after skin biopsies in a participant of the microneedling group. (a) Masson's trichrome staining; before and after treatment, ×20. (b) Verhoeff-Van Gieson staining; before and after treatment, ×20

had more participants who were very satisfied but not completely satisfied. The final difference between both groups was not statistically significant $P = 0.116$.

Histopathological evaluation

Figures 3 and 4 show histopathological results comparing ADSC-treated sides with placebo for both groups. Figure A1 (MN) and Figure A2 (CO_2 laser) provide detailed comparisons highlighting which intervention yielded the most significant changes.

Collagen bundles

In the MN-ADSC group, most participants initially exhibited mild collagen levels ($n = 10$, 71.4%). By the end of the study, there was significant progression, with four participants (28.5%) reaching moderate levels and 71.42% ($n = 10$) achieving marked collagen levels. Similarly, the CO_2-ADSC laser group started with most patients at mild collagen levels ($n = 12$, 92.31%) but showed a more pronounced improvement, with 92.31% ($n = 12$) reaching marked levels by the end of the study. Nonetheless, the difference between groups was not statistically significant ($P = 0.18$).

Elastin fibers

For elastin, the MN-ADSC group showed an increase from mild-to-moderate levels, beginning with 57.14% ($n = 8$) at mild levels and concluding with 85.71% ($n = 12$) at mild levels and one marked. In contrast, the CO_2-ADSC laser group started with 76.9% ($n = 10$) at mild levels and ended with 69.2% ($n = 9$) still at mild levels, but with an increase in the moderate category to 30.7% ($n = 4$). The Mann–Whitney test for elastin levels produced a *P*-value of 0.4, suggesting no statistically significant difference between groups.

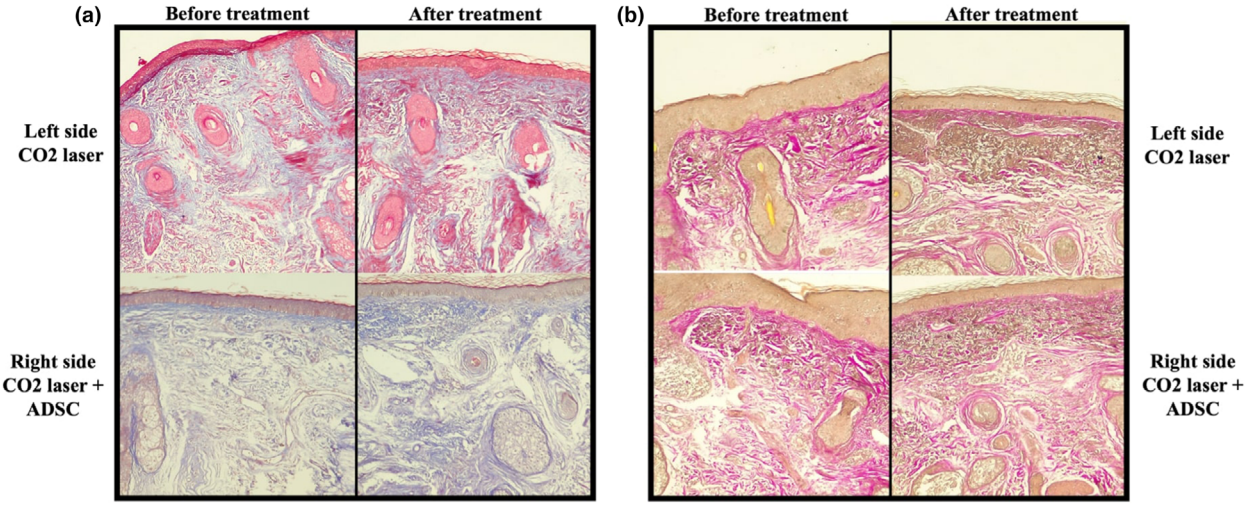

Figure 4 Before and after skin biopsies in a participant of the CO_2 laser group. (a) Masson's trichrome staining; before and after treatment, ×20. (b) Verhoeff-Van Gieson staining; before and after treatment, ×20

Epidermal thickness

Regarding epidermal thickness, all patients in the MN-ADSC group started at mild levels ($n = 14$) and progressed to moderate levels by the end of the study ($n = 13$). Similarly, the CO_2-ADSC laser group also began with all patients at mild levels ($n = 13$), most progressing to moderate levels ($n = 12$, 92.3%) by the study's conclusion. This difference was statistically significant ($P < 0.001$).

Solar elastosis

In the category of solar elastosis, the MN-ADSC group initially had the most participants at moderate and marked levels ($n = 4$, 28.5%, and $n = 9$, 64.2%, respectively). By the end of the study, most participants were at mild and moderate levels of solar elastosis ($n = 8$, 57.1%, and $n = 5$, 35.7%, respectively). The CO_2-ADSC group also began with most participants at moderate and marked levels ($n = 3$, 23%, and $n = 9$, 69.23%, respectively) but finished with the majority at mild levels ($n = 11$, 84.6%). This difference was not statistically significant ($P = 0.259$).

Adverse events

The participants in either group reported no serious adverse events during the study. Only occasional erythema or mild irritation occurred post-intervention, resolving within 1–2 days. No side effects or complications were noted in the 1-month follow-up.

Discussion

Combining ADSCs with CO_2 laser therapy resulted in better facial rejuvenation outcomes than combining ADSCs with MN. The CO_2 laser group exhibited more significant improvements in key objective measures, and histopathological evaluations and patient satisfaction further supported these findings.

Both interventions, when combined with ADSCs, improved skin texture and pore size, although these improvements were more pronounced in the CO_2 laser group. Subjective assessments further highlighted the superior efficacy of the CO_2-ADSC combination, with a higher percentage of participants reporting higher satisfaction rates. The improvement in solar elastosis observed in both groups, particularly in the CO_2-ADSC group, is a noteworthy finding. Although the difference was not statistically significant, the trend toward greater improvement suggests this combination might be especially effective in addressing this aspect of facial aging.

The improved outcomes for the CO_2 laser group are likely because of the combination of the fractional ablative laser's ability to induce controlled thermal damage, stimulate collagen production, and improve skin texture,[8] coupled with the regenerative potential of the ADSCs. In contrast, while MN also induces a wound-healing response, it lacks the same level of thermal and ablative effects that may be required for optimal rejuvenation. Notably, both interventions demonstrated excellent safety profiles, with only mild transient side effects observed.

Several studies have explored the efficacy of combining stem cell conditioned medium with nonsurgical facial skin resurfacing modalities such as MN and CO_2 laser. El-Domyati et al.[5] investigated the use of amniotic fluid-derived MSC conditioned medium combined with MN in a split-face study. They reported a significant overall improvement in skin condition, with 70% exhibiting improvement, especially in skin texture. Similarly, Hoss et al.[9] evaluated the effects of umbilical cord blood-derived MSC-conditioned medium with CO_2 laser resurfacing, where most of the participants reported being very satisfied with the active intervention. Our study also found significant reductions in wrinkles and improvements in skin texture, highlighting the benefits of combining MSC interventions for enhanced anti-aging effects.

Lee et al.[10] conducted a study on an endothelial precursor cell-conditioned medium that was applied during MN sessions. The results indicated reductions in wrinkles and pigmentation, similar to the findings observed in our CO_2-ADSC combination group. Prakoeswa et al.[11] used an amniotic membrane-derived MSC-conditioned medium with MN. They reported improvements in wrinkles, pigmentation, and pore size, with a notable overall enhancement in skin appearance when using MN-ADSC compared to MN alone. Lastly, Wang et al.[12] investigated adipose-derived MSC conditioned medium applied via MN and indicated significant improvements in skin wrinkles.

All five studies consistently reported that combining stem cell-conditioned medium with facial skin resurfacing modalities significantly improves skin condition compared to controls. These findings align with our results, where the combination of ADSCs with CO_2 or MN was superior to CO_2 laser or MN alone.

The primary difference between these studies is the type of stem cells used and the specific resurfacing modality applied. While our study focused on ADSC combined with either CO_2 laser or MN, other studies utilized various sources of stem cells, including amniotic fluid, umbilical cord blood, and endothelial precursors. Despite these differences, the overarching conclusion remains consistent: the integration of stem cell-derived treatments enhances the efficacy of traditional skin resurfacing techniques, which was further demonstrated in a meta-analysis.[13]

This study makes a unique contribution to the literature by directly comparing these two application methods. Our research strengths include a double-blind design and comprehensive objective and subjective evaluations. However, there are limitations. Although we did not analyze the presence of exosomes or growth factors, they likely contributed to the therapeutic effects. Exosomes secreted by ADSCs play a key role in intercellular communication and tissue repair, and recent research shows they can activate regenerative pathways like Wnt/β-catenin.[14] Future studies should quantify these components in the culture medium to understand their impact on ADSC efficacy better.

Another limitation is the 1-month follow-up period, which captured early treatment effects but left uncertainty about long-term results. Extended follow-up studies are needed to determine if the improvements are sustained over time, providing a fuller understanding of both efficacy and safety.

While our findings show that combining ADSCs with CO_2 laser or MN improves key objective parameters, the clinical significance must be considered carefully. The use of ADSCs should balance patient goals, treatment costs, and the degree of improvement. Although ADSCs yielded measurable benefits, particularly with CO_2 laser, the modest changes with MN suggest that ADSC use should be tailored to individual patient needs and expectations.

Furthermore, harvesting and culturing ADSCs is invasive and costly, involving liposuction and cell culture. CO_2 laser alone is an effective, established treatment for skin rejuvenation, improving texture, and collagen. Adding ADSCs may be justified for patients with severe skin damage or those seeking enhanced results, such as solar elastosis. However, CO_2 laser alone may offer a more cost-effective and less invasive option for moderate improvements. The cost–benefit ratio should be tailored to patient-specific goals, and further research is needed to guide clinical decisions.

Conclusion

In our study, the combination of ADSC with CO_2 laser therapy outperformed the combination with MN in achieving facial rejuvenation. Patients treated with the CO_2 laser and ADSC showed more significant improvements in UV spots, wrinkles, elastin fibers, and epidermal thickness and higher overall satisfaction rates. These results suggest that ADSC-applied post-CO_2 laser treatment offers a more effective method for facial rejuvenation. Future studies with larger sample sizes and longer follow-up periods are needed to confirm these findings and further establish the therapeutic potential of ADSC in esthetic dermatology.

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Appendix A

Table A1 Description of the antibodies used for flow cytometry

Analyte	Antibody	Fluorochrome	Supplier
Hematopoietic			
CD14	Anti-CD14 IgG2a, κ	PE	Abd Serotec
CD34	Anti-CD34 IgG1, κ	PE	BD Biosciences
Mesenchymal			
CD105	Anti-CD105 IgG1, κ	FITC	Abd Serotec
CD73	Anti-CD73 IgG1, κ	PE	Invitrogen
CD44	Anti-CD44 IgG2a, κ	PE	Chemicon
Control	IgG2a, κ isotype	PE	BioLegend
Control	IgG1, κ isotype PE	PE	BioLegend

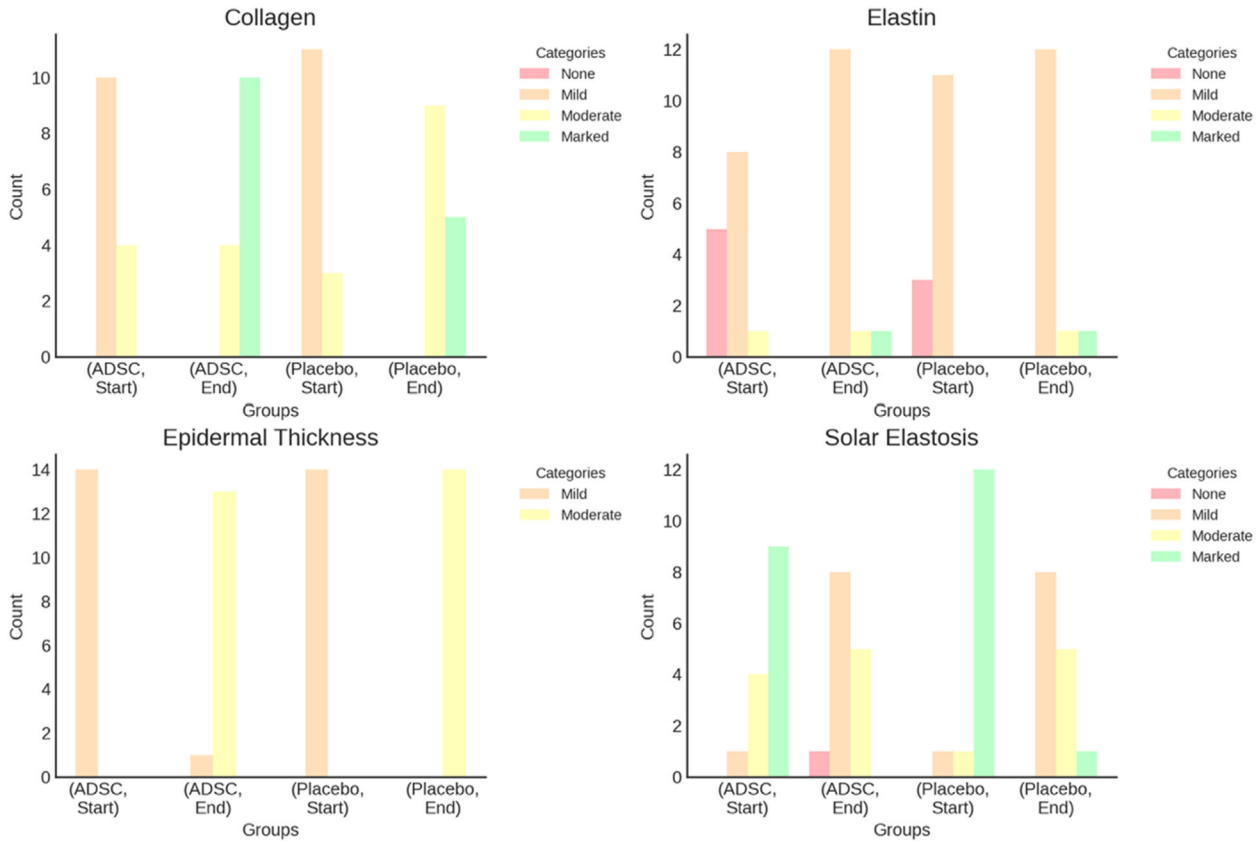

Figure A1 Comparison of pretreatment and posttreatment histopathological characteristics between the placebo-treated side and the ADSC-treated side in the microneedling group

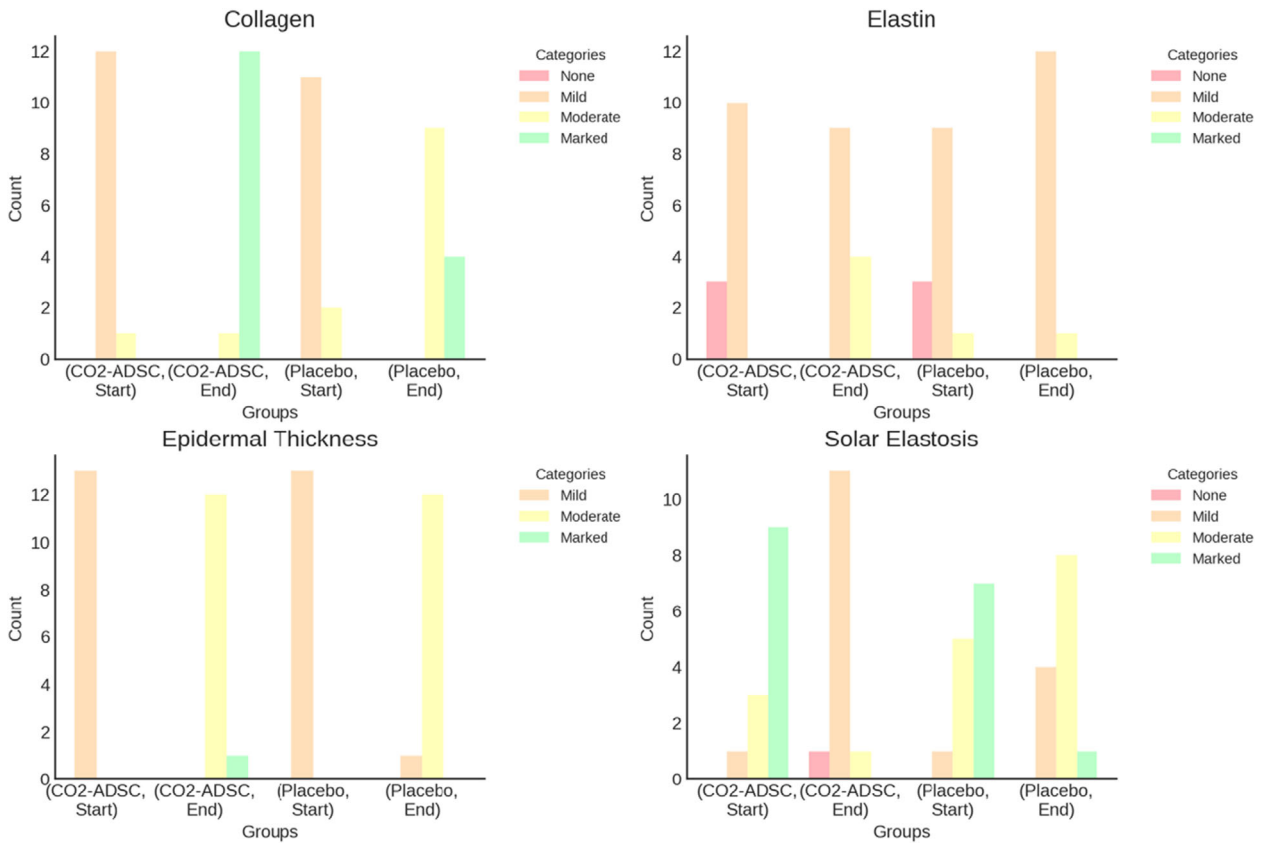

Figure A2 Comparison of pretreatment and posttreatment histopathological characteristics between the placebo-treated side and the ADSC-treated side in the CO_2 laser group