A Prospective Clinical and Instrumental Study on the Effects of a Transcutaneous Cosmeceutical Gel that is Claimed to Produce CO_2

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ABSTRACT

Carboxytherapy is the therapeutic use of carbon dioxide (CO_2) in its gaseous state. Since 1933, carboxytherapy has referred to either the subcutaneous injection of CO_2 or percutaneous application in a warm bath. The present clinical study was performed to determine if there were any changes in the dermis after the application of a transcutaneous gel, which is claimed to produce CO_2, and, if so, how these changes compared to those with CO_2 injection. Ten patients received transcutaneous treatment with the gel on one side of the face and the other side without any product was used as a control. We used videocapillaroscopy with an optic probe (VCSO) to evaluate the changes in the microcirculation of the skin. VCSO was performed for the treated right and untreated left ear lobes in each patient. VCSO was performed before treatment was started (VCSO1) and after 7 days of treatment (VCSO2).

A comparison of VCSO1 to VCSO2 showed an increase in the microcirculation, an increase in vertical and horizontal capillaries, and a reduction in the area of ischemia. These results are similar to those observed in other studies with CO_2 injection.

In conclusion, use of this transcutaneous CO_2 gel produced changes in the dermis similar to those observed with subcutaneous injection of CO_2.

INTRODUCTION

Over the past 25 years, carboxytherapy via CO_2 injection has been used to treat patients with different pathologies. When administered subcutaneously, CO_2 immediately diffuses at the cutaneous and muscular microcirculatory levels, resulting in higher tissue oxygenation and neoangiogenesis.[1] At the vascular level, CO_2 increases vascular tone and produces active microcirculatory vasodilatation as a result of its direct activity on arteriole smooth muscle cells. In addition, CO_2 promotes the Bohr effect, where the affinity of hemoglobin for oxygen decreases due to an increase in carbon dioxide, which means that extra oxygen is supplied to tissues that need it the most.[2] CO_2 also stimulates fibroblasts in connective tissues, which results in an increase in collagen.[3]

We previously studied the effects of carboxytherapy via CO_2 injection on the skin microcirculation. In those prospective studies, we used videocapillaroscopy (VCSO), by which capillaries in the skin are detected as being either vertical or horizontal, and changes in blood flow are measured by analyzing the diameter of the capillaries and their density (quantity of capillaries per mm^2).[4] We found increases in the percentage of vertical capillaries immediately after the first session using different doses (150cc and 50cc). Vertical capillaries significantly increased by $35.2 \pm 3.3\%$ per mm^2 in the area of injection (Fig. 1). After a week of carboxytherapy, the images showed an 8.2% decrease in vertical capillaries compared with previous images obtained immediately post-injection.[5]

The objective of the present study was to determine whether a gel that is claimed to release CO_2 into the skin actually increases blood flow in the capillary microcirculation, similar to the changes in the microcirculation observed with the injection of CO_2 into the dermis and subcutaneous tissue.

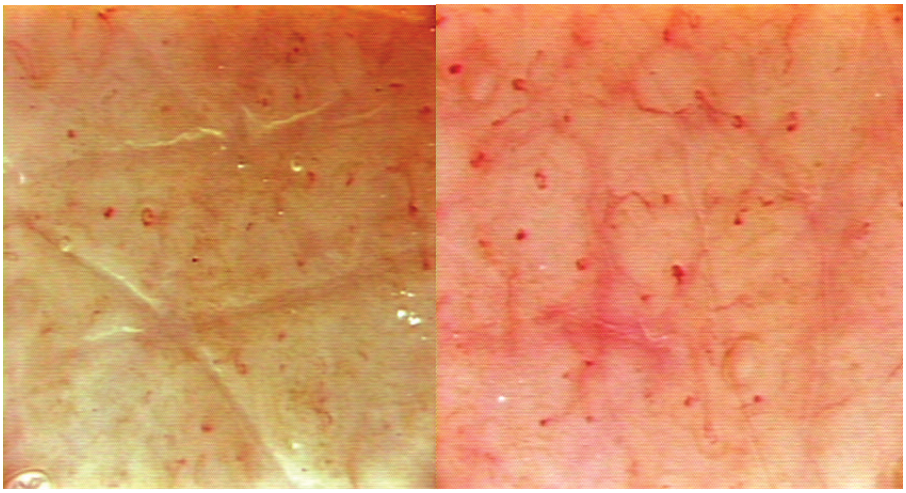

Fig. 1. Videomicroscopic images of a subject's skin before (left) and after (right) the subcutaneous injection of CO_2. There is a significant increase in capillary density. (200x)

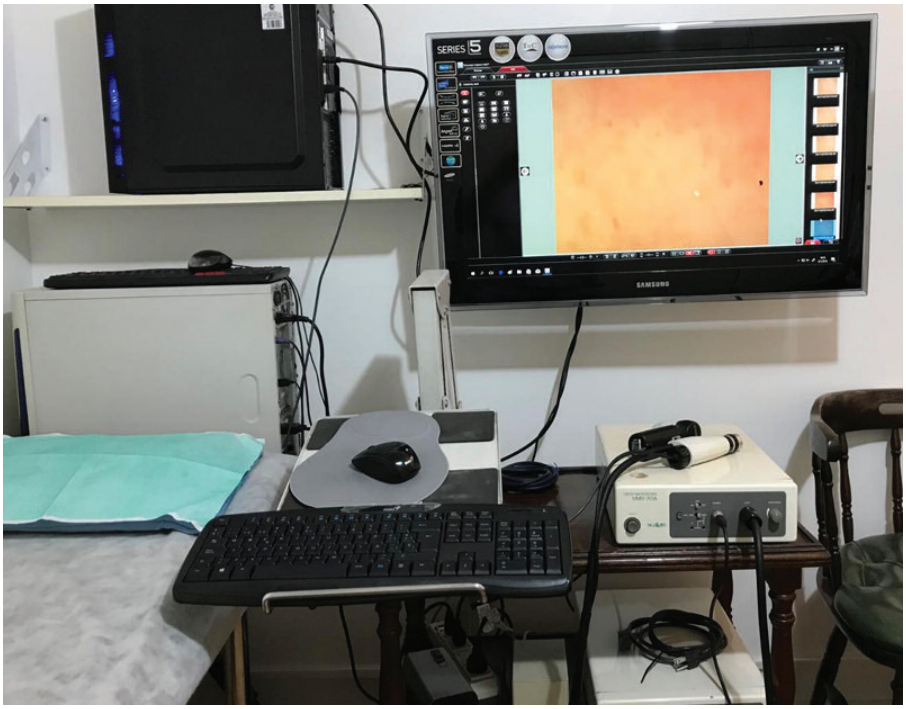

Fig. 2. Photograph of the instrumentation used. While the subject lay supine on the left, the operator had good access to their ear lobes for capillaroscopy. Images could be observed in real time and saved for later analysis.

MATERIALS AND METHODS

This was an observational, prospective and longitudinal study.

The subjects consisted of 10 volunteers (5 M/5 F; average age 38 ± 11 years) who were recruited from a private practice. They had skin type II and III according to the Fitzpatrick classification. None of the subjects had a history of allergic reaction or sensitivity to skin-treatment products. All of the subjects were in good general health, with no skin diseases or conditions, such as psoriasis or eczema.

Exclusion criteria were as follows: women who were currently pregnant or nursing or who could potentially become pregnant; individuals who were using medications that may have affected the study results, such as anti-inflammatory agents including Ibuprofen, Naproxen, Aspirin, etc.; individuals who had had a skin-treatment procedure in the test area, such as a chemical peel, tattoo removal, etc., within 30 days of study initiation; individuals with an abnormal skin condition, such as inflammation, sunburn or scars in the test site that may have interfered with the evaluation; individuals who reported allergies or sensitivity to skin-care products; individuals who had undergone wax epilation of the face within 14 days prior to baseline; and individuals who were expected to receive excessive sun exposure (greater than 1 hour per

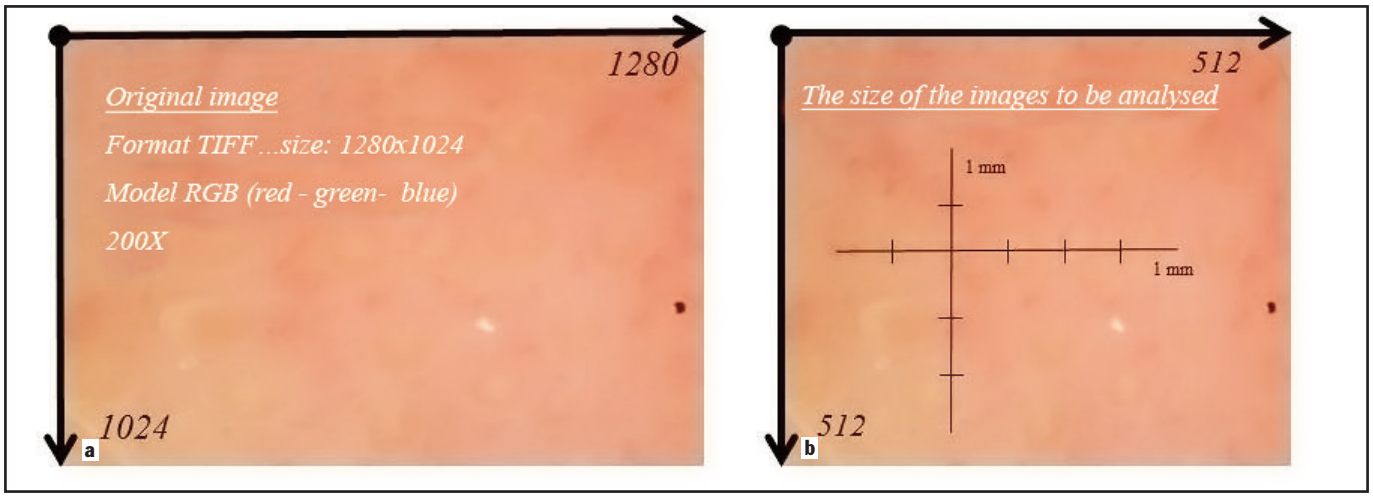

Fig. 3. Details of the images as acquired (a) and those used for image analysis (b).

day) during the study period.

All of the subjects gave their written informed consent to participate according to the Helsinki declaration.

The anterior surface of the ear lobe was used as the site for testing. The skin of the ear lobe is the same as that of any other part of the body. However, it offers the benefit of being small, which makes it possible to repeat treatment in the same area, and the capillaries are easily detected.

Observations were made using a videomicroscope with a 200x oil-immersion lens (ProScope HR, Bodelin Technologies, Wilsonville, OR) (Fig. 2).

Videocapillaroscopy (VCS) is a non-invasive technology that makes it possible to obtain in vivo images of the capillaries in the superficial dermis for studying the microcirculation in different areas of the skin and mucosal membranes. The analysis of static and dynamic images of capillaries by VCS with digital processing of images (DPI) is useful for evaluating the diagnosis and prognosis of diseases that compromise the microcirculation.[6,7]

In each volunteer, images of the right and left ear lobes were obtained on the same day at both the beginning of the study and when it was complete. The final images were obtained 7 days after application of the gel was complete. The average ambient temperature for image-capture was 23.04 ± 0.7 °C.

Subjects were treated with the topical gel CO_2Lift™ (Japanese patent 2016-20309; Lumisque Inc., Weston, FL). The manufacturer claims that this mixture of magnesium carbonate (Gel 1) and gluconolactone (Gel 2) provides results comparable to those seen with

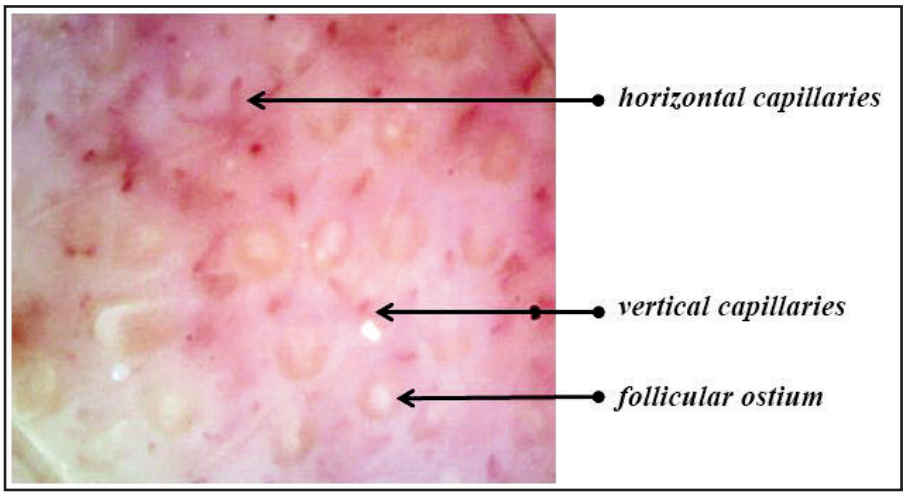

Fig. 4. Typical videomicroscopic image showing examples of horizontal and vertical capillaries.

Table I
Pre- and post-treatment measures of microvascularity in the treated right ear lobe

	Capillary diameter (µm)		Capillaries per mm²	
Patient ID	Before	After	Before	After
1	25.7	31.0	11	13
2	23.4	28.3	13	16
3	24.9	29.2	9	11
4	30.1	37.7	12	15
5	27.4	34.5	12	15
6	27.2	34.8	12	15
7	24.8	33.8	11	15
8	27.5	36.4	11	14
9	31.4	42.2	11	15
10	26.4	35.4	10	13
Mean	26.9	34.3*	11	14*

** Both mean capillary diameter and mean capillary density significantly increased with treatment (Wilcoxon test, $p \leq 0.05$).*

Table II
Pre- and post-treatment measures of microvascularity in the untreated left ear lobe

	Capillary diameter (µm)		Capillaries per mm²	
Patient ID	Before	After	Before	After
1	25.4	25.9	11	11
2	25.8	25.2	13	12
3	23.5	23.9	9	9
4	27.9	28.2	12	12
5	27.0	27.8	12	11
6	26.5	25.7	12	11
7	27.7	27.3	11	11
8	29.9	29.2	11	10
9	26.9	26.5	10	10
10	28.3	28.1	9	9
Mean	26.9	26.8	11	10.6

There was no significant change in either measure of microvascularity.

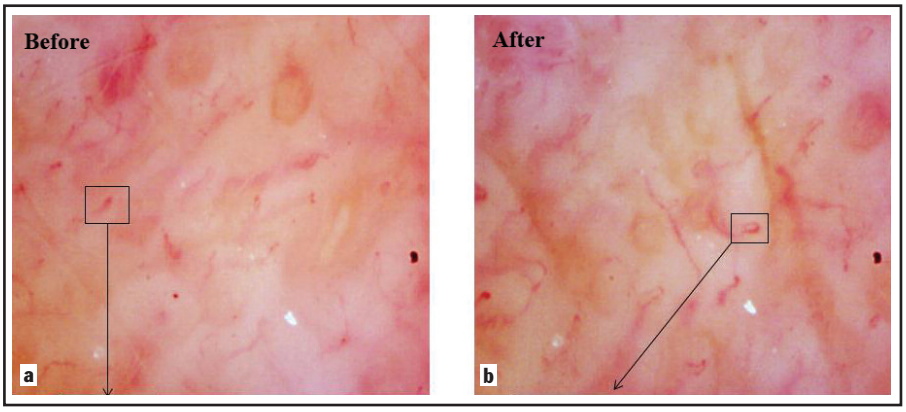

Fig. 5. Before treatment (a): average capillary diameter: 22.5µm, capillaries/mm²: 10. After treatment (b): average capillary diameter: 34.3µm, capillaries/mm²: 15.

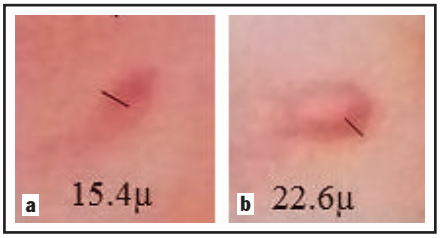

Fig. 6. Example of the increase in capillary diameter between before (a) and after (b) treatment.

CO_2 injection.[8]

Each volunteer was asked to apply the product to their right ear lobe every 48 h for a total of 5 applications, following the manufacturer's instructions. Each volunteer was provided with a means to record the date and time of application, and was instructed in writing how to prepare the product: i.e., mix the 2 component gels with a spatula for 1 min, and then apply the mixture to the right ear lobe for 1 h.

An average of 15 images of each ear lobe (both treated and untreated in each subject) were obtained at the beginning and end of the study. Images were captured in TIFF format (1280 x 1024); 512 x 512 images were used for analysis. Each image was treated by equalization (Figs. 3 and 4).[9,10]

RESULTS

Table I compares the results between before and after treatment on the treated side. There were significant increases in both the capillary diameter and the number of capillaries per mm² (Wilcoxon test, $p \leq 0.05$). By videocapillaroscopy, there was a 43.2% increase in vertical capillaries in the lobe of the right ear (treated area).

As shown in Table II, there were no changes in either the diameter or number of capillaries in the untreated left ear.

Pre- and post-treatment measurements for the lobes of the treated right ear and untreated left ear are shown in Figs. 5-9.

Figure 9 provides an example; the right ear lobe shows 18 capillaries/mm² and the untreated left ear lobe shows 13 capillaries/mm².

On average, the capillaries of the right ear lobes showed a 27.7% increase in blood flow, as reflected by capillary diameter, during the study period. During the same period, the density of capillaries increased by an average of 26.8%.

In a comparison of the treated right and untreated left ear lobes at the end of the study, the right ear lobes showed average increases of 28.2% in capillary diameter and 34.0% in capillary density.

DISCUSSION

Carboxytherapy by injection has been used to improve microcirculation in the dermis and subcutaneous tissue, increase the partial pressure of oxygen in the injected tissues (Bohr effect), stimulate the formation of collagen in the connective tissue of the skin, and restore the consistency of the extracellular matrix, which all help tissues to recover their normal physiology (biostimulation).[11,12] While this therapy is useful for treating various aesthetic pathologies (cellulite, facial aging, dark circles, stretch marks, scars, etc.),[13] it can also be used to treat varicose or diabetic ulcers, lesions on the limbs of diabetic patients, and deficiencies in the arterial microcirculation. There are also recent studies on its use in patients with burns.[14-16]

The main problem with carboxytherapy by injection is the pain that is caused by the introduction of gas into the skin. This pain is caused by the diffusion of CO_2 in the dermal and subcu-

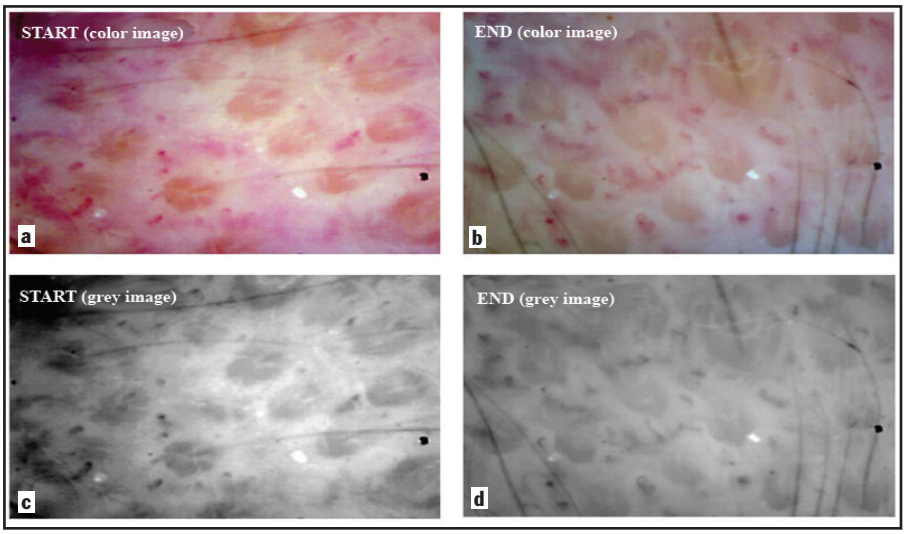

Fig. 7. Density of vertical capillaries. Before treatment (a): 13/mm²; After treatment (b): 16/mm². Mean capillary diameter. Before treatment (c): 19.5µm; After treatment (d): 28.0µm.

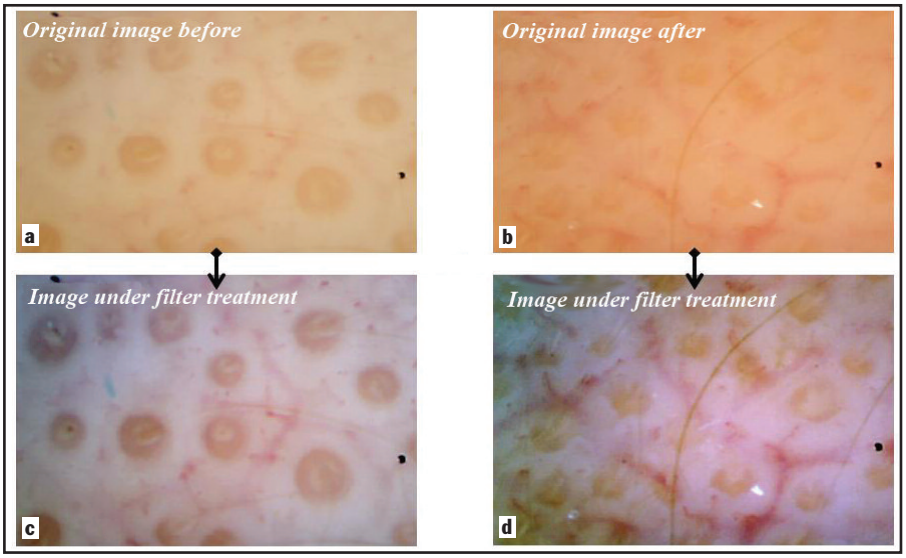

Fig. 8. Density of vertical capillaries. Before treatment (a): 12/mm²; After treatment (b): 16/mm². Mean capillary diameter. Before treatment (c): 21.6µm; After treatment (d): 27.9µm.

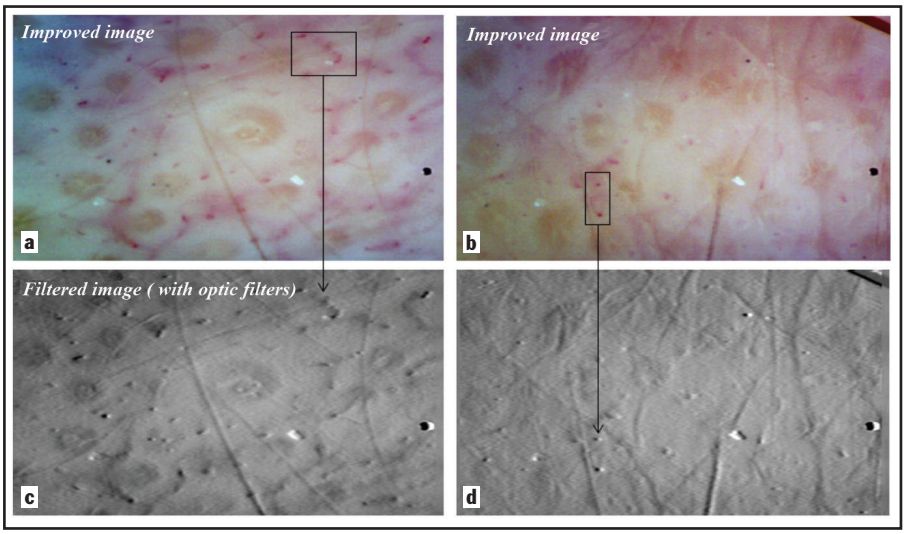

Fig. 9. Comparison of vertical capillary density between the treated and untreated earlobes. The density of vertical capillaries in the untreated left earlobe (13 capillaries/mm²) (b,d) was less than that in the treated right earlobe (18 capillaries/mm²) (a,c). Lower panels (c,d) show filtered images.

taneous tissue, which produces localized emphysema and barotrauma. This barotrauma induces the tissue-regeneration phenomena that are associated with the therapeutic use of CO_2.[17]

Thus, a product that could provide the benefits of CO_2 but without causing discomfort to patients, such as the present transcutaneous gel, would allow us to expand our treatment, increase the number of application sessions, and allow us to combine therapies in complicated cases to achieve longer-lasting, more effective, therapeutic results. The present results show that it is possible to achieve changes in the microcirculation with the transcutaneous application of a gel that produces CO_2 through a chemical reaction.[18]

CONCLUSION

This preliminary prospective study on the effects of a transdermal gel that is claimed to produce CO_2 are very encouraging with regard to stimulation of the cutaneous microcirculation. To confirm these results, further studies with a greater number of patients will be needed. STI

AUTHORS' DISCLOSURES

The authors have no conflicts of interest to disclose.

REFERENCES

1. Sinozic T, Kovacević J. [Carboxytherapy supportive therapy in chronic wound treatment] (in Croatian). Acta Med Croatica 2013;67 Suppl 1:137-41.
2. Tyuma I. The Bohr effect and the Haldane effect in human hemoglobin. Jpn J Physiol 1984;34(2):205-16.
3. Ferreira JCT, Haddad A, Tavares SAN. Increase in collagen turnover induced by intradermal injection of carbon dioxide in rats. J Drugs Dermatol 2008;7:25-30.
4. Thulesius O. Capillaroscopy with fiber optic video microscopy. VASA 1992 21;87-8.
5. Goldman M; Leibaschoff GH (eds): Cellulite Physiopathology and Treatment. Taylor and Francis, New York, NY, 2008.
6. Arpaia G, Cimminiello C, Milani M, et al. A new capillaroscopic assessment of microvascular damage vs Maricq classification in patients with isolated Raynaud phenomenon. Int Angiol 1989;8(3):129-33.

7. Cutolo M, Sulli A, Smith V. How to perform and interpret capillaroscopy. Best Pract Res Clin Rheumatol 2013;27(2):237-48.
8. Sakay Y, Miwa M, Oe K, et al. A novel system for transcutaneous application of carbon dioxide causing an artificial Bohr effect in the human body. PLoS One. 2011;6(9):e24137.
9. Piovella C, Del Guercio R. La video capillaroscopia a sonda ottica. Manuale de Microciurlazione per la clínica (in Italian). Minerva Medica 1995;51-84.
10. Fagrell B. Vital capillaroscopy. A clinical method for studying changes of skin microcirculation in patients suffering from vascular disorders of the leg. Angiology 1972;23:284-98.
11. Podgórna K, Kołodziejczak A, Rotsztejn H. Cutometric assessment of elasticity of skin with striae distensae following carboxytherapy. J Cosmet Dermatol 2017 Dec 6. doi: 10.1111/jocd.12465.
12. Pianez LR, Custódio FS, Guidi RM, de Freitas JN, Sant'Ana E. Effectiveness of carboxytherapy in the treatment of cellulite in healthy women: a pilot study. Clin Cosmet Investig Dermatol 2016;9:183-90.
13. Paolo F, Nefer F, Paola P, Nicolò S. Periorbital area rejuvenation using carbon dioxide therapy. J Cosmet Dermatol 2012;11(3):223-8.
14. Sinozic T, Katić M, Kovačević J. [Personalized approach to patient with chronic wound in family medicine](in Croatian). Acta Med Croatica 2016;70 Suppl 1:105-10.
15. Duraes EF, Durães EF, Durães Lde C, Carneiro FP, Lino Rde S Jr, Sousa JB. The effect of carbon dioxide therapy on composite graft survival. Acta Cir Bras 2013;28(8):589-93.
16. Brandi C, Grimaldi L, Nisi G, et al. The role of carbon dioxide therapy in the treatment of chronic wounds. In Vivo 2010;24(2):223-6.
17. Maia-Figueiró TL, Odashiro AN, Menezes GP, et al. Semi-quantitative histological analysis of the effect of intense pulsed light (IPL) and carbon dioxide (CO_2) intradermic injection on fibroblast and collagen proliferation in the skin of Wistar rats. J Cosmet Dermatol Sci Appl 2012;02(03):164-73.
18. Nishimoto H, Inui A, Ueha T, et al. Transcutaneous carbon dioxide application with hydrogel prevents muscle atrophy in a rat sciatic nerve crush model. J Orthop Res 2017 Nov 28. doi: 10.1002/jor.23817.