# PRELIMINARY PROSPECTY STUDY OF THE USE A COMBINATION OF GEL THAT PRODUCE TRANSCUTANEOUS CARBOXYTHERAPY IN THE TREATMENT OF SMALL FOOT DIABETIC ULCERS

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Diabetes mellitus, is a major worldwide health issue, afecting an approximated 382 million people. The global prevalence of diabetes is growing, and by 2035 around 592 million people will be impacted(1) There are nearly 29 million people with diabetes in the United States, and around 25% of people older than age of 65 have diabetes.

The occurrence of diabetes worldwide is expected to grow by 55% over the next 20 years, so this problem will only worsen; 75% of diabetic neuropathies are approximately distal symmetric polyneuropathy (DSP).(2)

When peripheral neuropathy develops, the annual rate of ulcer formation increases from 1% to greater than 7%. (3)

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia (high levels of glucose in the blood), which results from malfunctioning insulin secretion, faulty insulin action, or both (4,5,6). An insulin failure, in the long term, can cause damage to many organs and tissues, leading to life-threatening health complications such as neuropathy (7,8,9), cardiovascular

diseases (10), nephropathy (11-12), ocular diseases (13,14) and ulcerations (15–17).

In addition to those, the development of chronic nonhealing foot ulcerations (diabetic foot ulcer (DFU)) (18), which increases the risk of amputation (19), is a significant cause of mortality and the most important cause of hospitalization of diabetes patients (20,21)

It is estimated that 15-25% of DM patients develop DFU at least once in their lifetime.

Frequent complications of poorly controlled diabetes are diabetic foot ulcers, DFU, and they often become infected, described as diabetic foot infection (22)

In approximately 40% of diabetes-related foot ulcers, diabetes-related foot infections occur, producing significant morbidity.

When evaluating for a foot infection or osteomyelitis, it should be considered patient risk components (e.g., presence of foot ulcers more significant than 2 cm, uncontrolled diabetes, poor vascular perfusion, comorbid illness) (23)

In diabetic patients with ulcers, the three-year mortality rises from 13% to 28%

The need for an interprofessional team in the management of diabetic foot infections is highlighted by its pathophysiology

The compromised blood flow is a significant factor in diabetic food infections

According to the level of local trauma and microvascular disease, diabetic foot infections may vary from a straightforward case of cellulitis to full-blown gangrene.(24)

Osteomyelitis occurs in 15% of ulcers, and 15% of those will go on to require amputation.

60% of patients undergoing lower extremity amputation have diabetic foot ulcers as the underlying cause. After a lower extremity amputation, the five-year mortality jumps to 60%.

Every effort should be made to prevent Diabetic foot ulcers because they are antecedents to amputation and mortality

It is not easy to treat diabetic foot infections; the antibiotics usually cannot get to the infected area because the blood flow is compromised

The proper detection of pathogens causing the infection needs to be monitored. (25)

New molecular techniques, in recent years, have helped the bacterial identification and quantification.

Molecular techniques would be better to find the specific species hiding in the wound and select the proper antibiotics to diffuse from the skin.

The cure of a foot infection can take much longer in patients with diabetes mellitus than a non-diabetic.

There are several considerations in developing diabetic foot ulcers. The pathophysiology includes neuropathy, ischemia, nutritional dysfunction, and infection.

A foot suffering from PVD, peripheral vascular disease is more susceptible to ulceration and infection than a well-vascularized foot

The reduced perfusion efficiency in people with diabetes is caused by autonomic neuropathy, where there is fewer capillaries recruitment and blood shunting around capillary beds

Neuropathy also causes a diminished sensation and a loss of sweat and oil glands, leading to dry cracking skin and a diminished neuroinflammatory response to noxious stimuli (26).

Moreover, glycosylation of tendons causes stiffening and shortening, generating foot deformities (claw toes, hammertoes) and Achille's tendon stiffening, increasing pressure on the forefoot(27).

Once an ulcer develops, the physical exam should include a thorough screening for peripheral vascular disease and sensory neuropathy and an evaluation of the depth and severity of the ulcer.

it is essential to consider all the previous treatment strategies, together with the importance of what they wear on their feet, socks, and shoes

Transcutaneous oxygen tension should be at least 40 mmHg for a regular wound healing

A high number of patients with diabetes mellitus will have lower levels (28).

A team of multiple professionals is a must to treat diabetic foot ulcers, DFU

The immune-compromised status in patients with diabetes and foot ulcers, plus the low tolerance for fighting cover-up infections and the ability to heal effectively, make it a complex combination to treat

Optimal glycemic control and diabetic regimen, proper local wound care, pressure redistribution on the ulcer by mechanical offloading, infection management, and ischemia must be supervised.

PAD (Peripheral arterial disease) and signs of limb ischemia should be diagnosed

Revascularization is the key to managing ischemic diabetic lesions (29)

Treatments considering early revascularization in diabetic patients with any degree of limb ischemia is also a way to prevent diabetic foot ulcers

The objective of wound healing is re-epithelialization, or wound closure, an essential part of the healing process. This process is accelerated by the migration of cells at the margin towards the center of the wound (30).

A key aspect of fibroblasts is that they provide the contractile forces to bring the wound edges together, for which their migratory capability is crucial

Diabetic wounds deteriorate from reduced neovascularization (31,32).

One of the major difficulties in achieving wound closure in patients is the absence of epithelial migration

Although the extracellular environment of diabetic wounds is hypoxic, which increases the permanence and expression of HIF-1α typically, studies have shown that HIF-1α levels are reduced in diabetic wounds (33)

In hyperglycemic situations in the diabetic wound, the function and stability of HIF-1α are decreased by high levels of glucose and reactive oxygen species (ROS). The formation of new vessels is smaller, and wound healing is inadequate.

High glucose conditions were shown to perturb keratinocyte functions and negatively impact the resolution of inflammation and re-epithelialization (34)

These findings suggest that high glucose conditions in diabetic patients may impair fibroblast functions necessary for the remodeling of the dermal layer during wound healing (35,36)

Macrophages derived from circulating monocytes recruited to the wound play essential roles in the inflammatory and proliferative phase of wound healing. A study reported an increase in pro-inflammatory M1 macrophages and decrease in anti-inflammatory M2 macrophages in the serum of diabetic patients (37)

Diabetic wounds have been reported to have decreased production of pro-angiogenetic factors, giving rise to lower vascularity and capillary density. This could be partially attributed to the effect of high glucose environment on endothelial cell(38)

Taken together, there is substantial evidence to show that the high-glucose environment in diabetic patients impedes wound healing by altering the functions of keratinocytes, fibroblasts, macrophages, and endothelial cells during wound healing.

Following the chapter of Yuk Cheung Cyrus Chan Biomedical Technology Cluster HK China about Angiogenic response in wound healing "the most important physiological responses that dictate the healing process is angiogenesis (as well as vasculogenesis) and the mechanism associated with its malfunction during DM"

Angiogenesis is the creation of a new blood vessels from the present blood vessel network, described by the protrusion and development of capillary buds and sprouts of the vessels

Vasculogenesis is a physiological reaction that determines the blood vessel restoration. It refers to de novo formation of blood vessels including the differentiations of ECs from the progenitor cells

Ancillary complications associated with this endocrinal condition are also increasing.

Disturbance of the harmony in glucose homeostasis causes hyperglycemic status at the beginning of specific metabolic pathways. The persistence of the abnormal state leads to vascular insufficiency, nerve damages headed by ulceration in the lower extremity due to plantar compressions and foot abnormality. (39)

Any trauma at the affected site goes unnoticeable to the patient due to loss of sensation. In addition to the mentioned causes, diabetic foot ulcers are potentially modifying complications. Resistance to infection is considered the principal modulator of the pathophysiological image of diabetic foot lesions.

The healing and nonhealing nature of ulcers depend on the wound microbial populations and the extent of their pathogenicity.

The proper detection of pathogens causing the infection needs to be monitored (40)

The diagnosis of foot ulcers colonized by different bacteria is using conventional techniques.

New molecular techniques have helped discover bacterial identification and quantification in recent years. Molecular techniques would be better for finding the specific species hiding in the wound.

Diabetic foot ulcers are precursors to amputation and mortality, and therefore, every effort should be made to prevent them.

In our previous work on the use of injectable carboxytherapy in patients with diabetic ulcers, we had emphasized the importance of the treatment of diabetic ulcers (41)

DFU requires a systematic knowledge of the significant risk factors for amputation, frequent routine evaluation, scrupulous preventive maintenance, and the correction of peripheral arterial insufficiency.

Carboxytherapy refers to the subcutaneous injection of CO2 to improve microcirculation and promote wound healing because of the increase of oxygen.

Since optimal ulcer healing requires adequate tissue perfusion, Carboxytherapy consequently is useful in treating DFU.

Our previous prospective clinical study included 40 patients with DFU presenting different sizes and types of chronic ulcers

The treatment protocol included: wound cleaning, debridement, if necessary, antibiotics, blood sugar control, medication, healthy habits, no weight-bearing, and injectable carboxytherapy.

The results showed that this treatment that included carboxytherapy promoted wound healing and prevented amputation.

Our next challenge is to evaluate the treatment of small diabetic foot ulcers with a cosmeceutical product that produces CO2.

For that, we developed a prospective clinical research work in 10 patients with small DFU using applications of CO2 Lift gel for one month at the rate of three weekly sessions

In previous research of Dr. Leibaschoff, Dr. Coll found that using the combination of CO2 Lift gels creates microcirculatory changes equal to those observed when we injected CO2.(42)

We think that CO2 Lift gels, transcutaneous CO2, will have a beneficial effect promoting healing of the ulcer due to microcirculatory and PO2 increase in the treated area.

**Carboxytherapy** originally referred to the subcutaneous injection of CO2 to improve the microcirculation and thus promote wound-healing. The clinical use of CO2 is not new. The injection of CO2 gas, Carboxytherapy, is used in Argentina and France to treat peripheral arterial disease, mainly peripheral arterial occlusive disease (PAOD) of the lower limb.

When we inject CO2 subcutaneously, it immediately diffuses at the cutaneous and muscular microcirculatory levels, producing microcirculatory vasodilatation and improving flow through a direct action on arteriole smooth muscle cells Via the Bohr effect.

It also increases the tissue pO2 at the injection site;

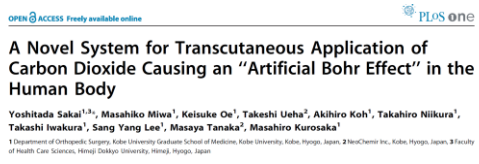
The Bohr effect refers to a decrease in the affinity of hemoglobin for oxygen due to an increase in CO2, which means that extra oxygen is available to other tissues (the

oxygen dissociation curve shifts due to changes in the concentration of CO2).

There is also a subsequent stimulation of fibroblasts and an increase in the quality of the extracellular matrix (ECM). CO2 can be used alone as a base treatment to help regeneration or in combination with other procedures to synergize the outcomes.

**Transdermal Carboxytherapy** (CO2 Lift gels) is a new treatment option that provides carbon dioxide (CO2 ) through the skin's superficial layers.

The CO2 promotes tissue oxygenation through the Bohr effect and contributes to regeneration in the wound area. Sakai et al.(Fig 1) reported that transcutaneous CO2 was beneficial for therapeutic purposes via increased blood flow and microcirculation as evaluated by laser Doppler and intensification of tcpO2 in ischemic tissues, providing evidence of the Bohr effect in vivo.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A Novel System for Transcutaneous Application of Carbon Dioxide Causing an "Artificial Bohr Effect" in the Human Body

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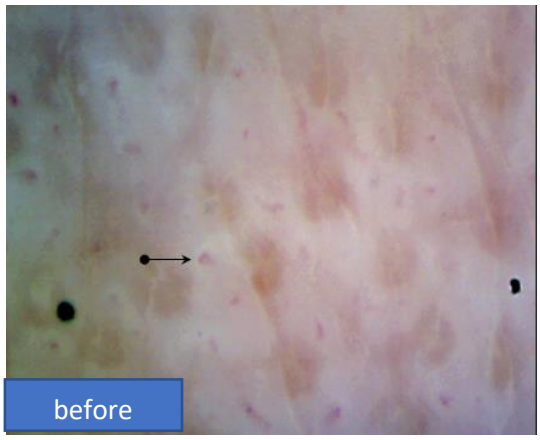
Fig 1

Leibaschoff et al. used video capillaroscopy to evaluate the effect of CO2 transdermal gel and found that it improves the microcirculation, comparable to the improvement in microcirculation observed after subcutaneous CO2 injection (Fig. 2).



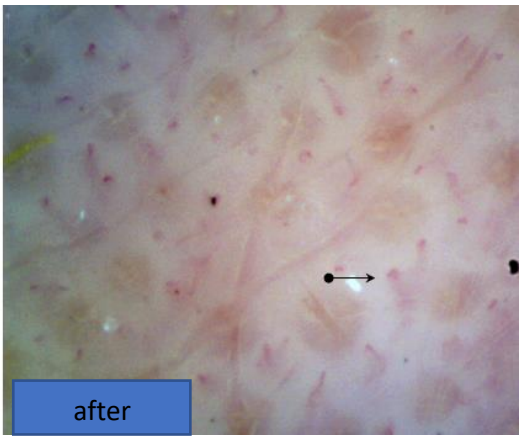




Fig 2

We decided to gather more data and evaluate the efficacy of transdermal CO2 gel to help wound healing through improvement in microcirculation and oxygen as the first objective in patients with small diabetic foot ulcers.

The secondary objectives were to evaluate the efficacy of transdermal CO2 in the treatment of small diabetic ulcers related to changes in quality of life and the patient's clinical satisfaction

The prospective clinical research work was done in 10 patients with small DFU and no infections with anaerobic germs or osteitis through applications of CO2 Lift gel for one month at the rate of three weekly sessions

The gel used here, CO2LIFT ® (Lumisque, Weston, FL, USA), is a commercial product mixture of two gels (Fig 3): one gel contains magnesium carbonate the other has gluconolactone. When we mix the two components, it generates CO2 for 45 minutes.


Fig 3

## Method

### PATIENT SELECTION

The patients selected for the study had small DFU in the feet, without any infection with anaerobic bacteria or osteitis

They gave their written informed consent and committed to following the instructions.

They received written detailed instructions about the use of the product.

Laboratory studies can be helpful in identifying malnutrition (pre-albumin), renal disease (BUN and creatinine), poor glycemic control (Hemoglobin A1C) and screening for osteomyelitis (CRP > 10 and ESR > 40).

When cultures are taken, it is important to get a deep tissue culture rather than a superficial swab because most wounds have superficial colonization which is not necessarily representative of the most important infectious cause

X-ray findings of osteomyelitis take four to six weeks to develop, therefore, if there is a high index of suspicion of osteomyelitis

The treatment was performed in four treatment cycles (three days a week, total of 12 days of treatment); each process included three days weekly gel applications in the ulcer areas.

The transdermal CO2 gel was prepared by mixing the respective pouches in a sterile warm glass bowl or container until homogenous coloration was achieved (Fig. 4).


Fig 4

The combination was applied over the wound area with a sterile single-use spatula, leaving a thick layer (Fig. 5 ,6).

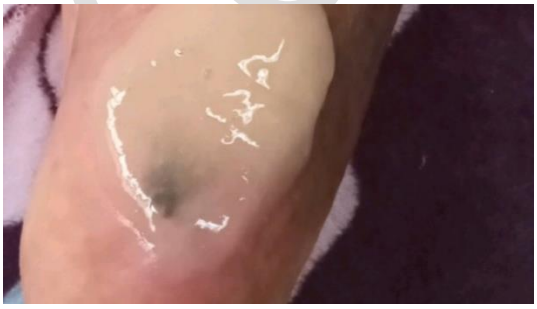


Fig5

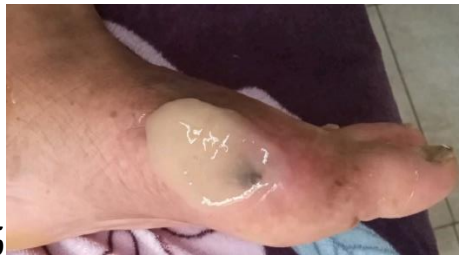


Fig 6

The gel was left in place for 45 minutes and then removed with the spatula

All the patients have the follow protocol:

Blood test

Bacteriology study of the wound

Antibiogram

Ecodoppler vascular multifrequency linear probe in the lower limbs

RX of the foot

Patient 1

Young 13-year-old, type 1 diabetic patient with a chronic lesion in the heel

Developing for four months.

( chronic wound because these a bearing area )

The patient does not present vascular disorders of the lower limbs or osteitis

Bacteriological study of the wound: finds a golden staphylococcus.

Blood tests: without particularities

Treatment: Fucidine 250 mg ..... 2 tabs .... 2 /day for two weeks.

Description of the wound

Picture 01 : Heel crack with superficial ulceration and limit by hyperkeratosis

Last picture : Complete healing with residual hyperkeratosis because the plantar skin is thick

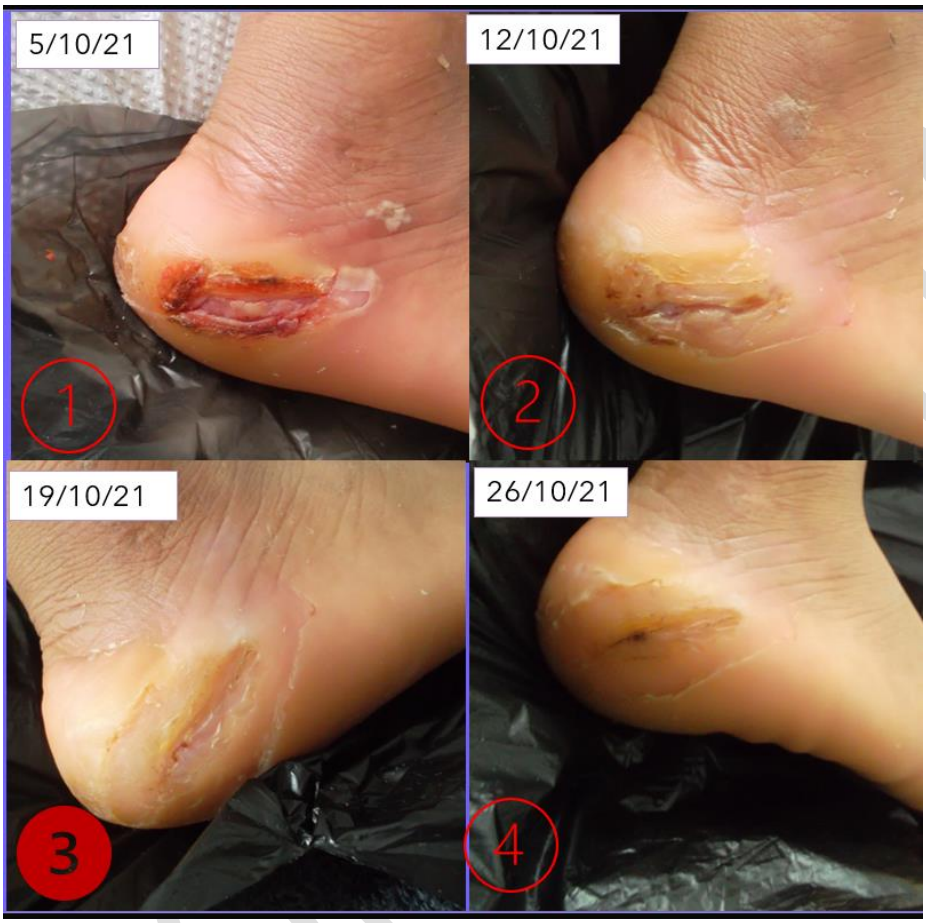



Fig7

Patient 2

54-year-old, type 2 diabetic patient on mixed and rapid insulin with a superficial wound over the external malleolus.

Lower limbs echo doppler: atheromatous plaques without hemodynamic repercussions.

RX: no osteitis  Bacteriological + for gram+

Blood test: good kidney and liver function

Fucidine 250 mg ..... 2 tabs /day for four weeks

<u>Description of the wound :</u>

Picture 01: Superficial Ulceration of the external malleolus

Picture 04: The budding sore has a red appearance, a tissue expression on the way to healing.

This aspect of the wound is a sign of good vascularization, which allows the migration and implantation of fibroblasts.fig 8

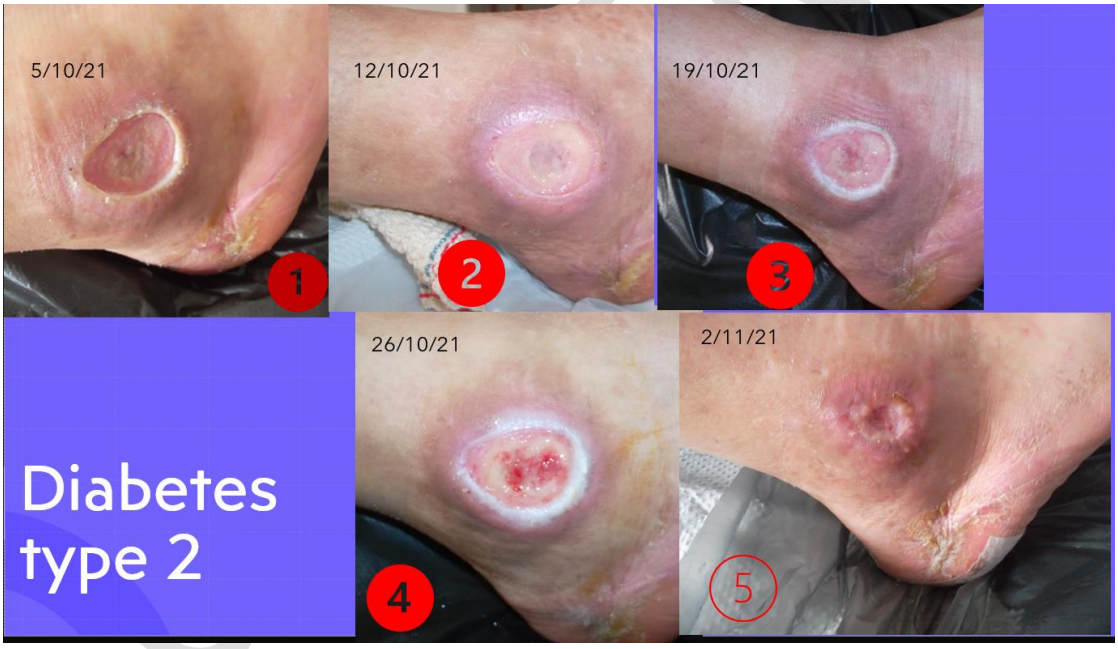



Fig 8

Patient 3

65-year-old patient with type II diabetes on insulin and lesion in the big toe

Echo Doppler No vascular Troubles

Blood test normal

Bacteriological: No germs isolated, but the patient was on Fucidine 250... 2 tabs; 2 / day for four weeks.

RX: No osteitis

<u>Description of wound :</u>

Picture 01: superficial ulceration and hyperkeratosis, sometimes called keratosis, is an abnormal thickening of the outermost layer of the skin

Picture 04 The wound: The healing is effective when the wound progresses at this stage. Indeed, a budding wound is a sign of optimal recovery of the vascularization. Fig 9

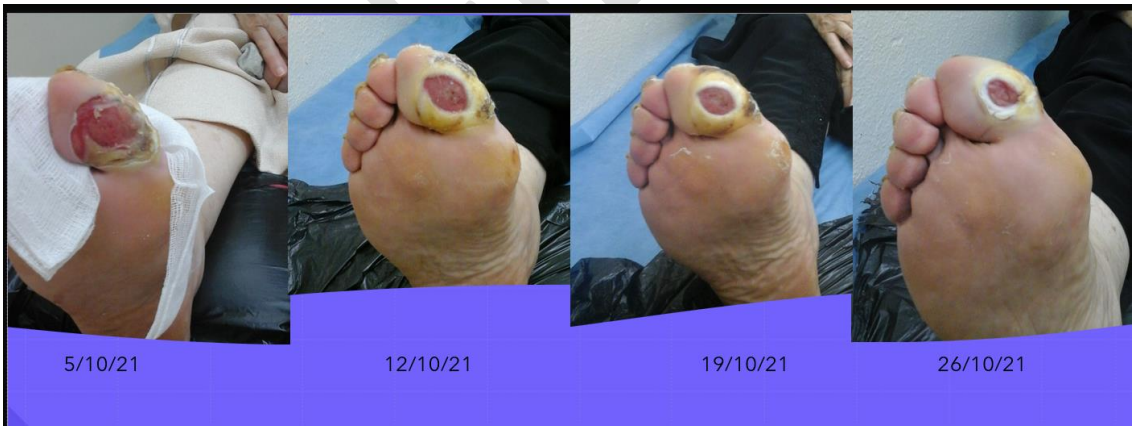


Fig 9

Patient 4

55-year-old type II diabetic patient on insulin with superficial ulceration next to the Achilles tendon caused by new shoes.

Lower limbs echo doppler: atheromatous plaques without hemodynamic repercussions

Blood test Normal

Bacteriological positive for Gram +

RX No osteitis

The patient is on Fucidine 250 mg 4 tabs per day for four weeks.

<u>Description of the wound :</u>

Picture 01: Superficial ulceration close to the Achilles tendon, shoe sore.

Picture 4: complete healing Fig 10

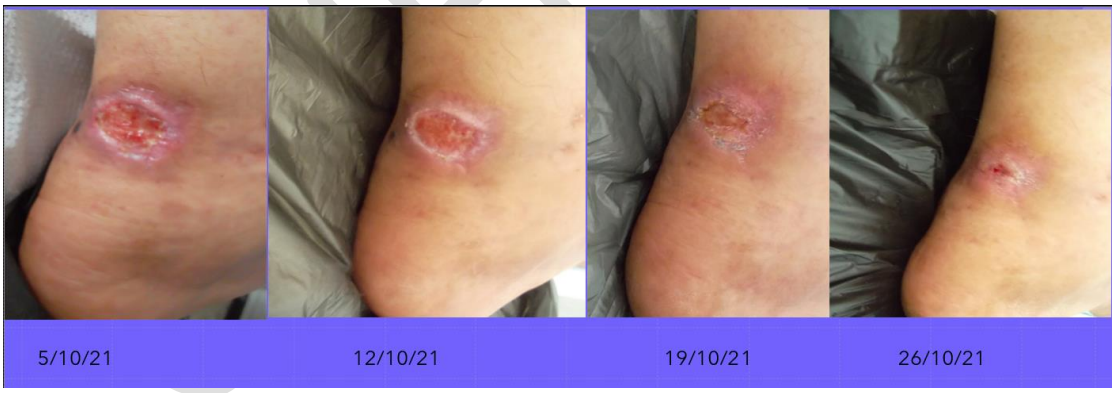



Fig 10

Patient 5

63-year-old patient, diabetic type 2 under insulin treatment, with a wound in the inner edge of left foot, following trauma in a construction site.

ECHO doppler: Arterial network of the infiltrated lower limbs without significant lesion, absence of anomalies of the venous network.

Blood test: HB: 11.1 Fasting glycemia 1.04 HBA1C = 7.56 Vit D: 16.1

Bacteriological: + staphylococcus aureus.

The patient is on Metronidazole 500mg and ciprofloxacine 500mg for five weeks.

Iron supplements 1tab/day and VIT D 200 000UI 1 ampoule every 15 days

RX No OSTEITIS

Description of the wound :

Picture 01: The injury has a red appearance, reflecting the presence of tissue on the way to healing, a sign of good vascularization, which allows the migration and implantation of fibroblasts.

Last picture: wound showing a good healing Fig 11

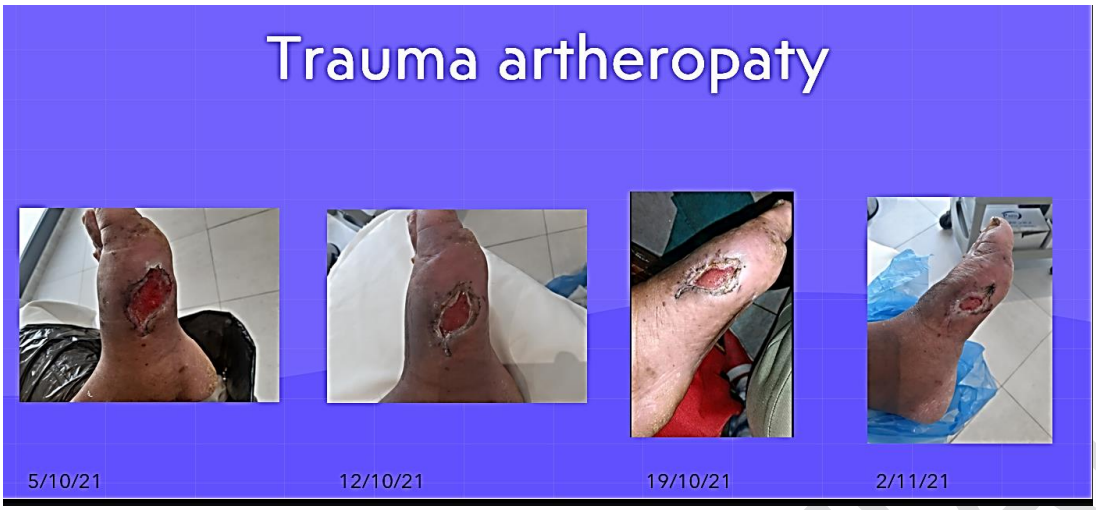


Fig 11

Patient 6

56-year-old patient with type 2 diabetes on insulin with a lesion in the inner lateral foot

Echo doppler: normal

Blood Test: normal

Bacteriological:+ gram +

The patient is on Fucidine 250 mg, 4 tabs per day for four weeks.

RX No osteitis

<u>Description of wound :</u>

Picture 01: A weeping wound, an exuding phase can follow a redness and release a translucent liquid on the surface of the skin

Last picture : Wound in the process of good healing Fig 12

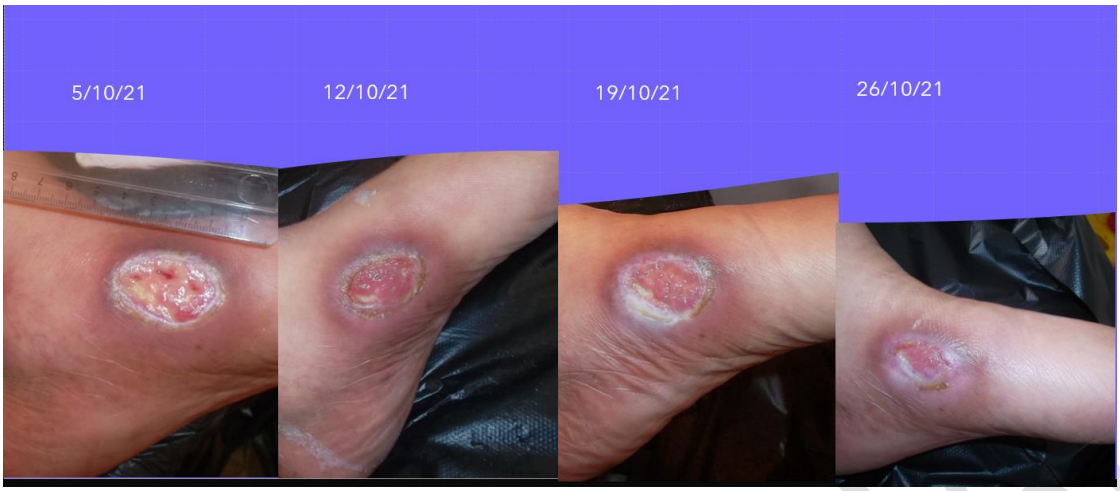


Fig 12

Patient 7

64-year-old patient with type 2 diabetes on insulin, with a lesion in the supra malleolar inner side

Echodoppler negative

Blood test normal

Bacteriological + for gram+

The patient is on Fucidine 250 mg, 4 tabs per day for four weeks.

RX no osteitis

<u>Description of wound :</u>

Picture 01: A weeping wound, an exuding phase can follow a redness and release a translucent liquid on the surface of the skin

Last pictures: Wound in the process of healing, signs of re-epithelialization Fig 13

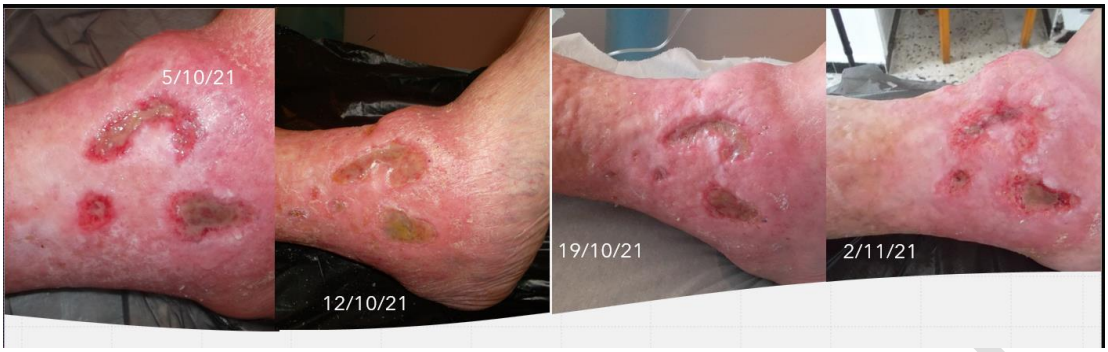


Fig 13

Patient 8

52-year-old patient with type 2 diabetes on insulin with superficial ulceration with a lesion over the toes after hot water burn

Echodoppler normal

Blood test Normal

Bacteriological + Gram+

The patient is on Fucidine 250 mg, 4 tabs per day for four weeks.

RX no osteitis

Description of wound :

Picture 01 : Clean ulcer and budding

Last pictures : Wound in the process of healing, signs of re-epithelialization Fig 14

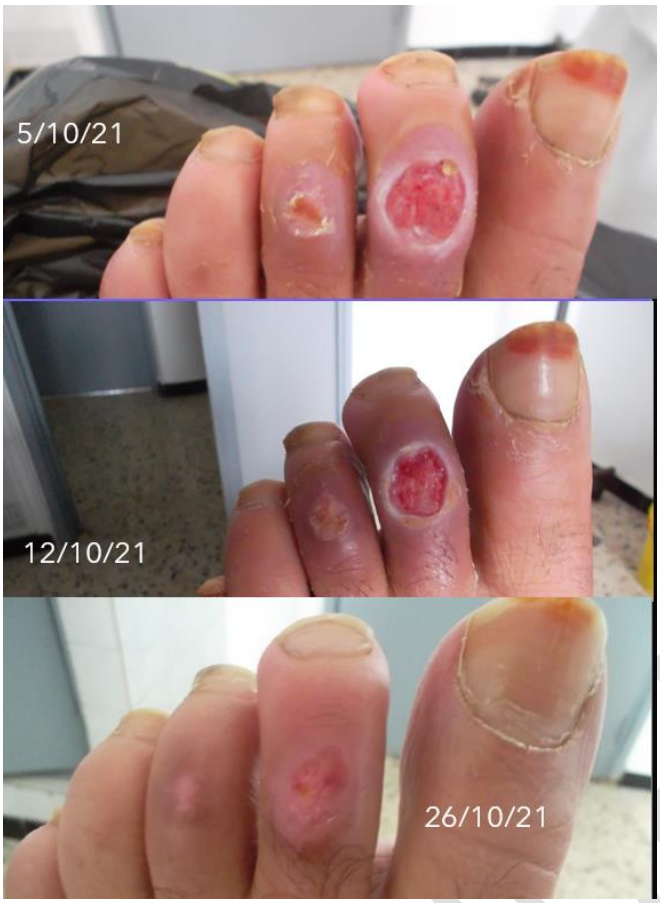



Fig 14

Patient 9

67-year-old patient with type 2 diabetes on insulin with varicose ulcer and a lesion over the external malleolous

Echodoppler Diffuse atheromatous arterial disease. Venous insufficiency in the great saphenous and Boyd perforant.

Blood test Normal

Bacteriological + STF

The patient is on cefazoline 1 gr, one injection per day, and metronidazole 500 1 tab twice a day for five weeks.

RX no osteitis

<u>Description of wound :</u>

Picture 01: Chronic varicose ulcer

Picture 02: A weeping wound, an exuding phase can follow a redness and release a translucent liquid on the surface of the skin Fig 15

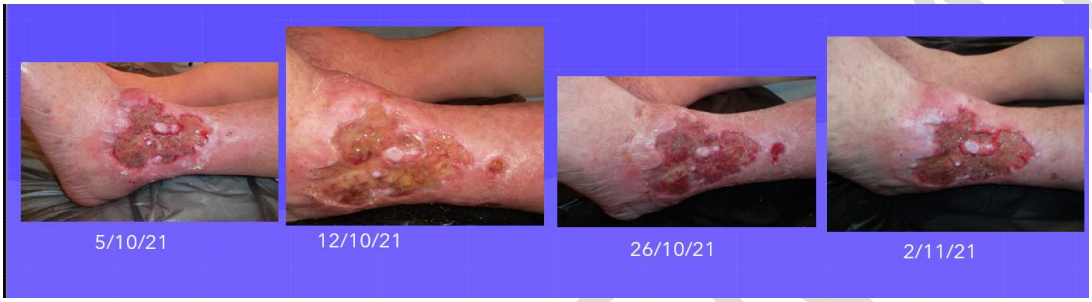



Fig 15

Last picture: in this case, the evolution is prolonged because it is associated with venous insufficiency

Patient 10

55-year-old patient with type 2 diabetes on insulin with a small ulcer in the big toe produce by inappropriate shoes

Echodoppler negative

Blood test normal

Bacteriological negative but the patients was under Fucidine

The patient is on Fucidine 250 mg,2 tabs twice per day for two weeks.

RX no osteitis

Description of wound :

Picture 01: Superficial ulcer.

Last picture : Complete healing with residual hyperkeratosis Fig 16

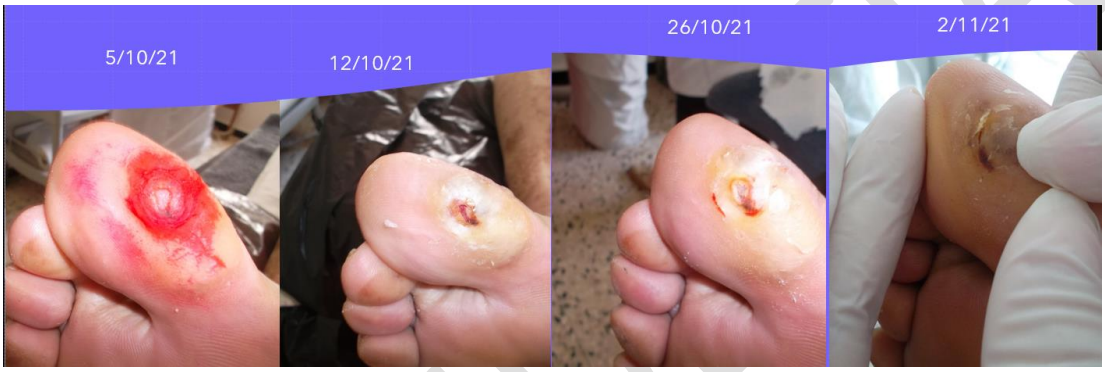


Fig 16

Conclusions

Continuous and complete clinical trials on DFU treatments have been done, including over 500 studies testing therapies such as debridement, oxygen, platelet gel, human amniotic membrane, laser, shockwave, vacuum-assisted closure, erythropoietin hydrogels, pulsed RF energy, mesenchymal stromal cells

The 85% of DFU are estimated to be preventable with an appropriate medical evaluation, supportive care, well-fitting shoes, and prompt treatment of wounds.

Additionally, physical activity and exercise decreased ulcer occurrence(44)

The prospective study was carried out during a short time (one month) in 10 diabetic patients with small ulcers on the feet (DFU) to show a new tool for treating small ulcers and avoiding severe complications.

Even though the number of patients was short, and we expected good results, we confirmed the outcomes in the study.

The results showed a stimulating effect; 100% of the participants evolved well.

A new non-invasive treatment for home routine has an opportunity to become an important armament in the treatment of small ulcers from DFU

The next challenge will be to enlarge the number of patients and conduct comparative studies with other treatments.



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