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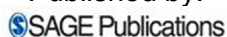
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Use of Nanotechnology-Designed Footsock in the Management of Preulcerative Conditions in the Diabetic Foot: Results of a Single, Blind Randomized Study

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The Difoprev system constituted by a sock loaded with nanocapsules containing a hydrating agent in the diabetic foot is tested. A total of 30 neuropathic outpatients with foot anhydrosis were randomized into group A, treated with the application of the sock with the nanocapsules, and group B wearing only the socks without the nanocapsules. Patients were blindly evaluated with a clinical score, hygrometry, transepidermal water loss, skin temperature, and skin hardness at baseline and after 6 weeks. No difference between the

groups emerged at baseline. Although group B showed no changes at the end of the treatment, group A significantly ($P < .05$) improved in all the parameters evaluated. No adverse events were recorded in both groups during the study. The use of hydrating agents carried by nanocapsules-loaded socks is safe and effective for the neuropathic diabetic foot.

Keywords: diabetic foot; nanotechnologies; ulcer; measurements; skin hydration

Foot ulceration is the most important and prevalent complication of diabetes mellitus and represents the most frequent cause of lower limb amputations in these patients, determining an excess of risk that is 20 times higher compared with general population.^{1,2} The costs of the management of this pathology, frequently complicated by infections that require admission of the

patients and use of parenteral antibiotic therapy regimens, are probably the most relevant among those related to diabetes and its complications.^{3,4}

The pathogenesis of foot ulceration is well understood. It is recognized that in the presence of peripheral neuropathy the effect of repetitive trauma leads to foot ulcers.^{5,6} All the components of diabetic neuropathy are involved in the pathogenesis of ulceration: sensory neuropathy reduces the ability to perceive external sensation as dangerous; motor neuropathy induces deformities in the foot that predispose to traumas; and autonomic neuropathy reduces the ability to sweat in the feet because of the sympathetic denervation of the sweat glands, which consequently atrophy, resulting in severe anhydrosis of the foot accompanied by dry skin and fissuring, WHICH represent the first lesion followed by a ulcer.^{7,8} Anhydrosis accompanies with hyperkeratosis due to localized hyperpressure and is the most typical sign of peripheral neuropathy; both of them are considered preulcerative conditions.^{9,10}

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Conflict of interest: None.

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Prevention of anhydrosis is dependent on the local application of moisturizers on the skin of the foot and the leg. This has proven to be an effective measure to prevent the ulcers, though it is also dependent on the effectiveness of the moisturizing agent and the compliance of the patient.¹¹

Recently, a biomedical application of the nanotechnologies made possible to design and to realize a tool that allows both to maximize the moisturizing action and to bypass the patients' compliance. This study reports on the application of the footsock.

The Difoprev System

The Difoprev (LVM Technologies, Bologna, Italy) system is based on a technology derived from the nanostructured lipid carriers, which has been demonstrated to be highly effective as a vehicle for both cosmetic and dermatological preparations. Its high-loading capacity, its physical and chemical long-term stability, and the triggered release are features that are all important for exerting its action.¹²

It consists of a sock made of a special synthetic fiber, polyamide fiber, which is smooth and does not irritate the skin, and which can be loaded with the microcapsules containing the active moisturizer because of negative/positive surface charges interaction. The sock is recharged with the nanocapsules at each washing; the washing detergent contains single-dose dispensers. The dispensers are manufactured as blisters, and in each box, there are enough washing units for 1-month.

The moisturizing agent is a nanoemulsion of liposomes, made by a core of lipids (phosphatidylcholine) coated by the glycoprotein secreted by the bacterium *Pseudoalteromonas antarctica*, that thrives in low-temperature environments and acts like an antifreeze.^{13,14} This protein, along with other proteins found in microorganisms, is able to coat liposomes, protecting them by surfacting agents, so that they increase their solubility and disposability.¹⁵ The liposomes, positively charged, can exert a range of activities in the skin. They interact with the extracellular lipid junctions in the stratum corneum (SC) and isolate the deeper layer from the outer ones. It is also reported that the liposomes have a positive activity on fibroblasts functions.¹⁶

Once the socks are worn the contained microcapsules are constantly released to the patient for all the time the sock is in contact with the skin. Each charge lasts for 36 hours, which thus eliminates the need to moisturize skin, whereas maximizing the time of application of the active agent.

This aim of this study was to test the safety and the effectiveness of the application of a system, that is, the socks coated with microcapsules or active socks to treat the preulcerative conditions of the diabetic neuropathic foot that are known risk factors for chronic ulceration.

Materials and Methods

All the diabetic outpatients attending our foot clinic during the months of June and July, 2005 were screened using the following inclusion criteria: they should have peripheral neuropathy, as defined by the American Diabetes Association,¹⁷ and they should have bilateral anhydrosis of the foot, identified with clinical examination and confirmed with the Neuropad (MIRO gmbh; miro Verbandstoffe, Wiehl-Drabenderhöhe, Germany) test, as described by Bilen et al.¹⁸

The exclusion criteria used were the presence of active ulceration in the foot, peripheral arterial disease, defined as an ankle-brachial pressure index <0.9, therapy with β -blockers, serum creatinine >2 mg/dL, and any local or systemic condition potentially interfering with skin structure and function.

After obtaining the informed consent according to the protocol approved by the Ethics committee of our hospital, patients were randomized into 2 groups by means of a computer-generated randomization list: group A was treated with the application of the active socks, according with the indication of manufacturers for 6 weeks, whereas group B wore just socks without the microcapsules for the same amount of time as those in group A. Patients and relatives were instructed on the procedures for the correct use of the active socks system. Patients in group B had to undergo an identical but sham recharging process of the socks to avoid bias. Patients of both groups were instructed in washing and recharging the socks every other day, according to the instruction of manufacturers. Two pairs of socks were given to each of them to guarantee the continuity of the study.

Patients were evaluated at baseline and after 6 weeks of continuous treatment for the following parameters: clinical conditions with a quantitative hydration score ranging from 1 to 5 (Table 1): skin moisture (Scalar Moisture Checker; STR, Scotts Valley, California), transepidermal water loss (TEWL; Vapometer; Delfin Technologies Ltd, Kuopio, Finland), skin temperature with infrared thermometry (La

Table 1. The Skin Hydration Score

Score	Definition	Characteristics
1	Anhydrosis	Absent sweating, fissuration, desquamation, anelastic, skin, absent pliability
2	Hypohydrosis	Sweating present only after stimulation, reduction or absence of dermatoglyphs, low pliability
3	Normohydration	Spontaneous sweating, normal pliability, and glyph, skin normoelastic
4	Hyperhydrosis	Excessive sweating after stimulation, hypertrophic dermatoglyphs, sporadic maceration in the interdigital areas
5	Maceration	Spontaneous excessive sweating, diffuse maceration, wet skin, eventual eczema

Crosse Technology, Cinisello Balsamo, Italy), and skin hardness with a durometer (Mod 3001/A; AFFRI, Induno Olona, Italy).¹⁹⁻²²

On healthy normal skin, the coefficient of variation (standard deviation/mean) of the tests used was <15% with repeatability ratio ranging from 0.77 to 0.84 for all the instruments. All the measurements were done by the same podologist (E.B.) who was blinded to the treatment being received by patients. The same procedure was carried out on all patients, and at all visits, the sites being the same point in the most severely affected foot, which was the summit of the plantar arch with the foot in a nonweight bearing position.

The feet of all subjects were photographed using a digital camera before and after the 6 weeks of treatment. Patients were instructed to do their regular activities and to record in a logbook any eventual adverse event, even if not apparently related to the treatment, and a telephone number was provided in case of emergency. Patients were also requested to keep all the remnants from the used boxes of the refills, so that a calculation about the effective usage of the system could be made retrospectively. Baseline clinical and instrumental characteristics of patients were compared with those of a group of sex-matched and age-matched healthy volunteers.

Data Analysis

Data were expressed as median (95% confidence interval) and analyzed using commercial software (Statview; SAS Institute, Gary, Illinois) on a personal computer. The statistical tests performed were the chi square test for the dichotomous variables and Mann-Whitney test for nonparametric data with $P = .05$ being the threshold for statistical significance.

Results

A total of 36 patients were screened between January and March, 2005 according to the inclusion and the exclusion criteria, but only 30 patients accepted to participate in the study and were actually enrolled and randomized in the 2 groups, their characteristics are listed in Table 2.

There were no differences between the 2 groups at baseline both for clinical features and objective assessment of the severity of the dishydrosis; patients were significantly different from healthy controls as shown in Table 3.

Figures 1A and 1B are photographs of a patient treated with the complete application of the active socks system. The change in the hydration of the skin, and the reduction of hyperkeratosis, and of wrinkles can be noted.

All the patients enrolled in both groups completed the study, and all patients returned the empty capsules used for recharging procedures. Calculations indicate that the recharging procedures were done every 2 (0.5) days, with no differences between the 2 groups.

No adverse events in both group A and group B were reported.

After 6 weeks of treatment, group A patients showed a statistically significant improvement ($P < .05$) in measured baseline parameters except in skin temperature. In comparison, the same values in group B were not significantly different ($P > .05$). These results are presented in Table 4. Skin temperature did not change significantly in group A before and after the study. Figure 2 shows the percentage differences from baseline values for each parameter for both groups.

Table 2. Characteristics of Patients Studied

	Group A	Group B	Controls	P
N. (DM1/DM2)	15 (2/13)	15 (1/14)	15	ns
Age, y	59.6 (13.8)	61.4 (15.5)	60.5 (11.4)	ns
Duration of diabetes, y	16.1 (9.0)	15.7 (6.9)	—	ns
HbA1c, %	8.6 (1.3)	8.9 (1.7)	—	ns

NOTE : DM1 = type 1 diabetes; DM2 = type 2 diabetes; ns = not significant; HbA1c = glycated hemoglobin.

Table 3. Baseline Clinical Features of Patients Studied Median (95% Confidence Interval)

	Group A	Group B	Healthy Normal Volunteers
Skin hydration score	1.1 (0.4)	1.2 (0.8)	3.5 (0.6) ^a
Skin hardness, IU	52.4 (7.8)	47.7 (7.1)	35.4 (8.8) ^a
TEWL, g/h/m ²	118.9 (45.7)	127.6 (49.8)	29.9 (16.4) ^a
Skin moisture, %	26.9 (14.8)	24.2 (13.6)	48.3 (12.2) ^a
Skin temperature, °C	34.1 (2.0)	33.8 (0.9)	34.1 (0.6)

NOTE: TEWL = transepidermal water loss.

^a $P < .05$ versus group A and group B.

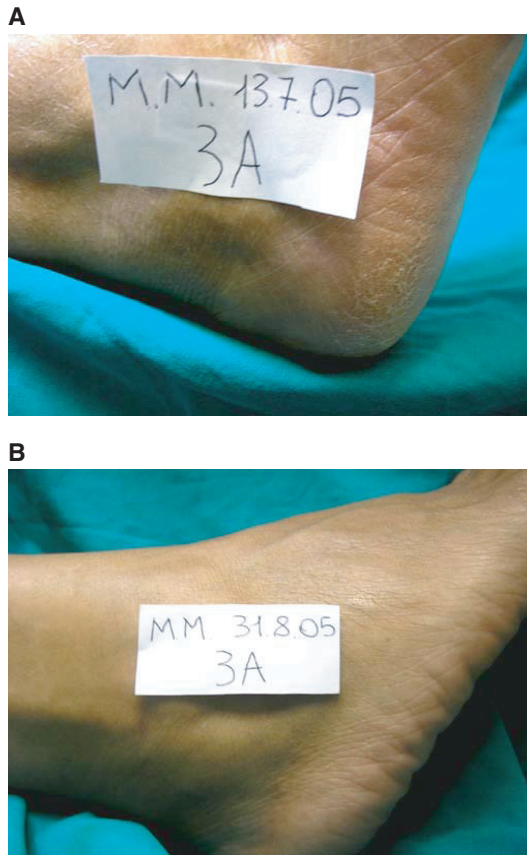


Figure 1. A case report of a patient treated with the Difoprev system. A, The patient's skin was dehydrated and anelastic, with a score of 1. The same patient after 6 weeks of treatment. B, The skin is normally hydrated and the score of 3 (refer Materials and Methods section).

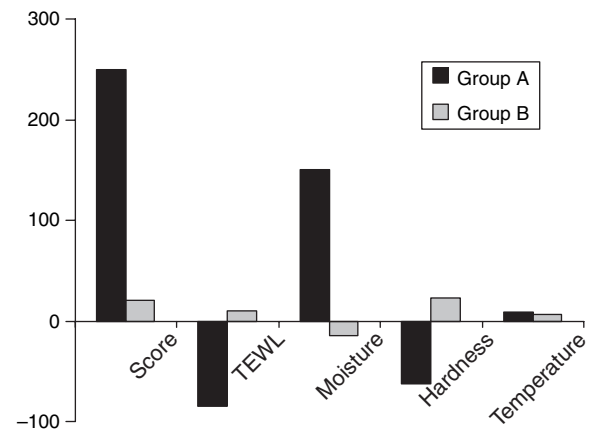


Figure 2. The rate of change from baseline in all the parameters was evaluated at the end of the study. An increase of 250% of skin hydration score, 150% of skin moisture, paralleled by a reduction of 85% of transepidermal water loss, and 62% of skin hardness was observed. All the differences observed in group A patients, except for skin temperature, were significant, whereas no significant changes were measured in the skin of patients in group B. We report the results of a small, single, blind randomized study. No adverse events were reported in either group A or in group B.

Discussion

The aim of this study was to demonstrate the safety of the charged socks to treat patients with diabetes with anhydrosis. The data show that the skin of patients who used socks carrying a microencapsulated hydrating agent for 6 weeks became significantly

Table 4. Results of the Clinical and Instrumental Evaluation in Both Groups Median (95% Confidence Interval)

	Group A Baseline	Group A+, 6 wk	Group B Baseline	Group B+, 6 wk
Skin hydration score	1.1 (0.4)	2.9 (0.8) ^a	1.2 (0.8)	1.4 (0.9)
Skin hardness, IU	52.4 (7.8)	26.4 (4.3) ^b	47.7 (7.1)	49.9 (6.9)
TEWL, g/h/m ²	118.9 (45.7)	44.1 (18.3) ^a	127.6 (49.8)	141.9 (57.2)
Skin moisture, %	26.9 (14.8)	49.8 (19.8) ^a	24.2 (13.6)	27.4 (15.7)
Skin temperature, °C	34.1 (2.0)	34.7 (1.7)	33.8 (0.9)	34.0 (2.1)

NOTE : TEWL = transepidermal water loss.

^a*P* < .01.^b*P* < .05 versus baseline.

more hydrated, retained more moisture, and was less hard compared with cohorts who were treated with sham socks. This could be potentially valuable in the treatment of diabetic foot disease that has an increasing prevalence globally.³ Prevention is crucial to avoid the progression of the disease from the early stages in which only predisposing conditions are present and the charged socks has the potential to aid this.²³

Well hydrated skin is more elastic and resistant to the shear stress obtaining the round-shaped margins of the foot during walking. This can reduce the risks of skin disruption and therefore ulceration.²⁴ The use of microencapsulated socks improved skin moisture and hardness, whereas no changes were measured in the other group which argues in favor of the properties of the socks.²⁵

We used different objective tests to assess hydration, water loss/barrier function, and hardness in this study. All these tests excepting skin temperature measurements were meaningful and are recommended for future studies with similar objectives.^{19,22}

In the patients of both groups the sudomotor activity was severely impaired as a consequence of autonomic neuropathy, we interpret the change reported to be the likely effect of the hydrating activity of the lipids carried as vehicles by the nanoparticles present in the activated system.

The way in which glycoprotein restores the normal hydration of the skin is probably related to its interaction with the SC of the epidermis that in the patients with diabetes has been found to be less hydrated and similar to that of senile patients.¹⁰

In our patients, the barrier function of SC was also restored with this approach as demonstrated by a highly significant reduction of TEWL in group A patients, whereas no significant change was measured in group B patients. This may be a reflection of the ability of nanoemulsion-containing lipids that

are reported to interact with the inner layers of the epidermis.²⁶

Positively charged nanoemulsions, like the one we used, are particularly effective because of the electric attraction between the particles and the negatively charged structures of the skin,²⁷ which may have potential benefits. Spreadability is also a key factor in enhancing the effectiveness of the emulsion; according to the Young-Dupre equation, the higher the extent of spreading, the higher is the attraction or adhesion between the emulsion and the skin.²⁶

There are other potential benefits. For example, the small size of the particles and large surface area of application may help maximize the interaction between skin and lipids coated with glycoproteins. The mode of application, that is, using socks, has potential benefits. Were this to be well accepted by patients, it would help management. It is reported that the combined presence of neuropathy and chronicity reduces the compliance of the patients^{28,29}; we suggest this aspect to be factored into future studies of such products.

The limitations are its small size and that it was a single blind study. Our aim was to test safety. This has been demonstrated, some clues as to potential benefits have also been shown. Future studies should be double blinded with an aim to determine the efficacy of the socks over a longer time period.

The results of this preliminary study permit the conclusion that the Difoprev system is safe and permits hydration of the neuropathic foot skin in patients with diabetes.

References

1. Diamant AL, Babey SH, Hastert TA, Brown ER. Diabetes. The growing epidemic. *Surgeon*. 2007;5:219-230.
2. Prompers L, Huijberts M, Apelqvist J, et al. High prevalence of ischaemia, infection and serious comorbidity in patients

- with diabetic foot disease in Europe. Baseline results from the Eurodiale study. *Diabetologia*. 2007;50: 18-25.
3. Boulton AJ, Vileikyte L, Ragnarson-Tenvall G, Apelqvist J. The global burden of diabetic foot disease. Review. *Lancet*. 2005;366:1719-1724.
 4. Reiber GE, Raugi GJ. Preventing foot ulcers and amputations in diabetes. *Lancet*. 2005;366:1695-1703.
 5. Urbancic-Rovan V. Causes of diabetic foot lesions. *Lancet*. 2005;366:1678-1679.
 6. Pecoraro R. Chronology and determinants of tissue repair in diabetic lower extremity ulcers. *Diabetes*. 1991; 40:1305-1313.
 7. Low VA, Sandroni P, Fealey RD, Low PA. Detection of small-fiber neuropathy by sudomotor testing. *Muscle Nerve*. 2006;34:57-61.
 8. Low PA, Benrud-Larson LM, Sletten DM, et al. Autonomic symptoms and diabetic neuropathy. A population-based study. *Diabetes Care*. 2004;27:2942-2947.
 9. Yosipovitch G, Hodak E, Vardi P, et al. The prevalence of cutaneous manifestations in IDDM patients and their association with diabetes risk factors and microvascular complications. *Diabetes Care*. 1998;21:4506-4509.
 10. Sakai S, Kikuchi K, Satoh J, Tagami H, Inoue S. Functional properties of the stratum corneum in patients with diabetes mellitus: similarities to senile xerosis. *Br J Dermatol*. 2005;153:319-323.
 11. Johnston MV, Pogach L, Rajan M, et al. Personal and treatment factors associated with foot self-care among veterans with diabetes. *J Rehabil Res Dev*. 2006;43: 227-238.
 12. Muller RH, Petersen RD, Hommoss A, Pardeike J. Nanostructured lipid carriers (NLC) in cosmetic dermal products. *Adv Drug Deliv Rev*. 2007;59:522-530.
 13. Sung JK, Joung HY. Cryoprotective properties of exopolysaccharide (p-21653) produced by the Antarctic bacterium, *Pseudoalteromonas antarctica* KOPRI 21653. *J Microbiol*. 2007;45:510-514.
 14. Nevot M, Deroncelé V, messner P, Guinea J, Mercadé E. Characterization of outer membrane vesicles released by the psychrotolerant bacterium *Pseudoalteromonas antarctica* NF₃. *Environ Microbiol*. 2006;8:1523-1533.
 15. de la Maza A, Codech L, Lopez O, Parra JL, Sabes M, Guinea J. Ability of the exopolymer excreted by *Pseudoalteromonas antarctica* NF₃ to coat liposomes and to protect these structures against octyl glucoside. *J Biomater Sci Polym Ed*. 1999;10:557-572.
 16. de la Maza A, Parra JL, Congregado F, Bozal N, Guinea J. Interaction of the glycoprotein excreted by *Pseudoalteromonas antarctica* NF₃ with phosphatidylcholine liposomes. *Colloids Surf A Physicochem Eng Asp*. 1998;137: 181-188.
 17. Boulton AJ, Vinik AI, Arezzo JC, et al. American Diabetes Association. Diabetic neuropathies: a statement by the American Diabetes Association. *Diabetes Care*. 2005;28:956-962.
 18. Bilen H, Atmaca A, Akcay G. Neuropad indicator test for the diagnosis of sudomotor function in type 2 diabetes. *Adv Ther*. 2007;24:1020-1027.
 19. Hoedlftke RD, Bryner KD, Horvath GG, Phares RW, Broy LF, Hobbs GR. Redistribution of sudomotor responses is an early sign of sympathetic dysfunction in type 1 diabetes. *Diabetes* 2001;50:436-443.
 20. Piaggese A, Romanelli M, Schipani E, et al. Hardness of plantar skin in diabetic neuropathic feet. *J Diabet Complications*. 1999;13:129-134.
 21. Pi-Chang S, Hong-Da L, Shyh-Hua EJ, Yan-Chiou K, Rai-Chi C, Cheng-Kung C. Relationship of skin temperature to sympathetic dysfunction in diabetic at-risk feet. *Diabetes Res Clin Pract*. 2006;73:41-46.
 22. Levin J, Maibach H. The correlation between transepidermal water loss and percutaneous absorption: an overview. *J Control Release*. 2005;103:291-299.
 23. Jeffcoate WJ, Harding KG. Diabetic foot ulcers. Review. *Lancet*. 2003;361:1545-1551.
 24. Boulton AJ. The diabetic foot: from art to science. The 18th Camillo golgi Lecture. *Diabetologia*. 2004;47: 1343-1353.
 25. Verdier-Sévrain S, Bonté F. Skin hydration: a review of its molecular mechanisms. *J Cosmet Dermatol*. 2007; 6:75-82.
 26. Yilmaz E, Borchert H. Effect of lipid-containing, positively charged nanoemulsion on skin hydration, elasticity and erythema – an in vivo study. *Int J Pharm*. 2006; 307:232-238.
 27. Rojanasakul Y, Wang LY, Bhat M, Glover DD, Malagna CJ, Ma JKH. The transport barrier of epithelia: a comparative study on membrane permeability and charge selectivity in the rabbit. *Pharm Res*. 1992;9:1029-1034.
 28. Wu SC, Armstrong DG. The role of activity, adherence, and off-loading on the healing of diabetic foot wounds *Plast Reconstr Surg*. 2006;117 (suppl 7):248S-253S.
 29. Johnston MV, Pogach L, Rajan M, et al. Personal and treatment factors associated with foot self-care among veterans with diabetes. *J Rehabil Res Dev*. 2006;43: 227-238.