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Case Report

Neuropathic diabetic foot ulcers treated with cerium dioxide nanoparticles: A case report

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ABSTRACT

Wound healing in diabetes is frequently impaired and its treatment remains a challenge. The ability of topical application of cerium (Ce) dioxide nanoparticles (CNPs) to accelerate wound healing in an animal model provides a rationale to develop this technology for use in humans affected by traumatic injury, diabetes and burns. We first described a case report of successful topical treatment of neuropathic diabetic foot ulcers with novel gel containing CNPs. The CNPs has bacteriostatic activity, anti-inflammatory properties and can penetrated into the wound tissue and reduced oxidative damage therefore protect regenerative tissue, suggesting a therapeutic potential for topical treatment of diabetic foot ulcers.

1. Introduction

The "diabetic foot" is a group of syndromes in which neuropathy, ischaemia, and infection lead to tissue breakdown. Lower extremity ulceration is a common complication for patients with diabetes with a lifetime risk as high as 25% [1]. Diabetic foot ulcers (DFU) lead to some form of amputation in 20% of patients and are associated with higher morbidity and mortality [2]. Approximately 60% of all DFU result from neuropathy; of these, half are related to peripheral arterial disease [3]. DFU tend to occur most commonly on the plantar weight bearing surfaces of the foot underneath the pressure point. Complications of DFUs, such as infections and gangrene, frequently lead to hospitalisation and to extensive tissue destruction or amputation and impaired quality of life, incur a significant cost economically, socially and psychologically [4,5]. As many as 15% of people with diabetes will develop DFU and that 85% of non-traumatic amputations in diabetics are preceded by a foot ulcer [6]. A study in Scotland observed that 68% of diabetic patients who had an amputation died within 5 years [7]. Even when ulcers are healed, >50% will have a recurrence after 3 years [4].

Treatment of DFU is good wound care, including debridement of devitalised tissue, offloading, optimising diabetic control and nutritional intake, use of antibiotics to treat infection and multidisciplinary input [8–10]. Characteristics of an ideal wound dressing [11]:

- Creates microclimate for rapid healing;
- Prevents dehydration (of the wound);
- Permeable to oxygen;
- Absorption of blood and exudate;
- Protects against secondary infection;
- Offers sufficient mechanical protection to wound but is non-adherent;
- Is non-toxic, non-allergenic, and non-flammable;
- Does not shed material into wound;
- Conforms to anatomical contours and resists tearing;
- Its properties remain constant in a range of temperatures and humidities;
- Accepts and releases medication;
- Is cost effective.

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Table 1
Gradation criteria for assessment of ulcer [27].

Parameters for Assessment	Gradation criteria			
	0	+	++	+++
Size	No discontinuity of skin/mucus membrane	¼ of previous area of the ulcer	½ of previous area of the Ulcer	> ½ of previous area of the ulcer
Pain	No pain	Localized pain during movement but relieved on rest	Localized pain even during rest	Localized pain even during rest and also towards other side
Edge	Adhere edge	Smooth, even and regular edge	Rough, irregular edge	Angry look
Smell	No smell	Bad smell	Tolerable, unpleasant smell	Foul and intolerable smell
Discharge	No discharge/Dry dressing	Scanty, occasional discharge/Little wet dressing	Often discharge needs daily dressing	Profuse, continuous discharge needs frequent dressing
Floor	Smooth, regular with granulation tissue/No need for dressing	Rough, regular, mild discharge, less granulation tissue/needs Dressing	Unhealthy, less granulation tissue/needs daily dressing	Unhealthy, no granulation tissue

Table 2
General laboratory instrumental examination.

HbA1c, %	Glucose, mmol/l	Total protein, g/l	Albumins, g/l	Glucosuria	X-rays of the foot	Doppler examination
9,1	10,2	69,1	42,2	–	No evidence of osteomyelitis were found	Stenotic lesions of main lower limb arteries not found

Table 3
The dynamics of wound healing.

	Baseline	Week 1	Week 2	Week 4
Size		++	++	–
Diameter, мм	0,8–0,9	0,4–0,5	0,3–0,4	–
Depth, мм	1,7–1,8	0,9–1,0	0,1	–
Pain	+	0	0	0
Edge	0	0	0	0
Smell	+	0	0	0
Discharge	+	+/0	0	0
Surface	+	0	0	0
	Fig. 1	Fig. 2	Fig. 3	Fig. 4

The bacteriostatic properties of cerium were first recognised at the end of the 19 t h century [12]. The first systematic analysis of these effects was published in 1947 [13,14] when cerium was tested against a panel of 39 bacterial species across 16 genera, including *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Cerium nitrate was an effective bacteriostatic agent against the whole spectrum of bacteria, but was pH dependent, with the greatest effect at slightly acidic pH values [14].

Cerium (Ce) oxide nanoparticles (CNPs) have shown promise as a therapeutic application due to their antioxidant auto-regenerative ability and low toxicity [15]. The electronic structure of CNPs at the nanoscale lead to their antioxidant activity. Both large surface-area-to-volume ratio with the reduction in particle size [16] and ability to reversibly switch between $Ce^{3+}$and Ce^{4+}- present on the surface [17] results in the formation of oxygen defects in the crystal lattice that act as “reactive sites” or “hot spots” for free radical scavenging [18].

This is an advantageous way of regenerating reduced CNPs and eliminating the reactive oxygen species (ROS). Now CNPs have been considered to possess catalase-mimetic activity [19] and superoxide dismutase-mimetic activity [20]. CNPs due to self-regenerative ROS scavenging property have been used in different areas of medicine where pathologies are associated with excessive oxidative stress and had radiation-protective effects [21], anti-inflammatory properties [22–25], neuroprotective effects [26].

Therefore we firstly reported a case report of topical CNPs application on neuropathic DFU healing.

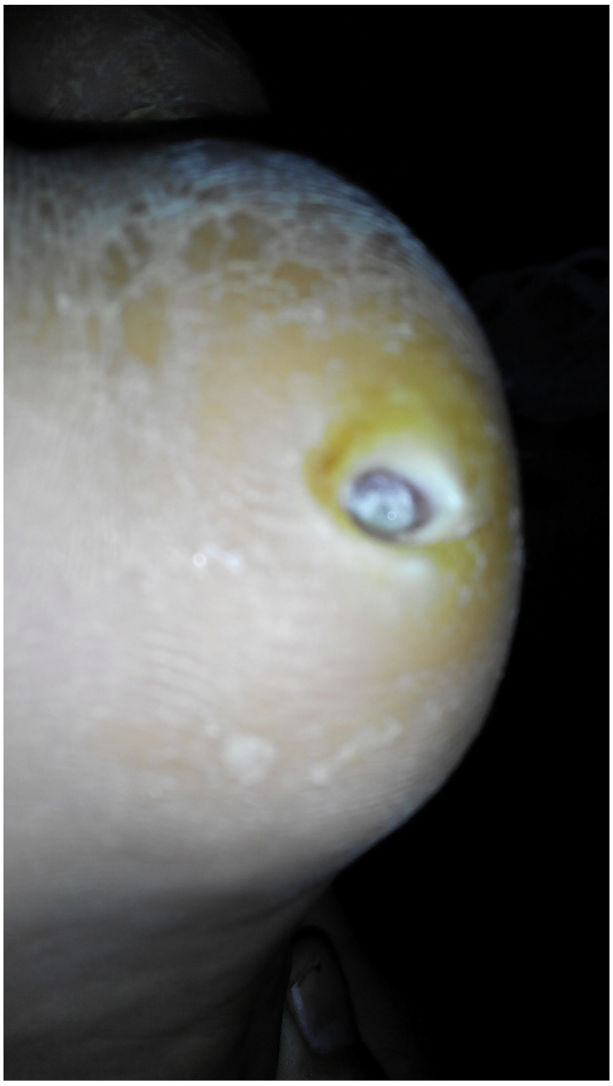

Fig. 1. Dynamics of wound healing-Baseline Dia 0.8–0.9.

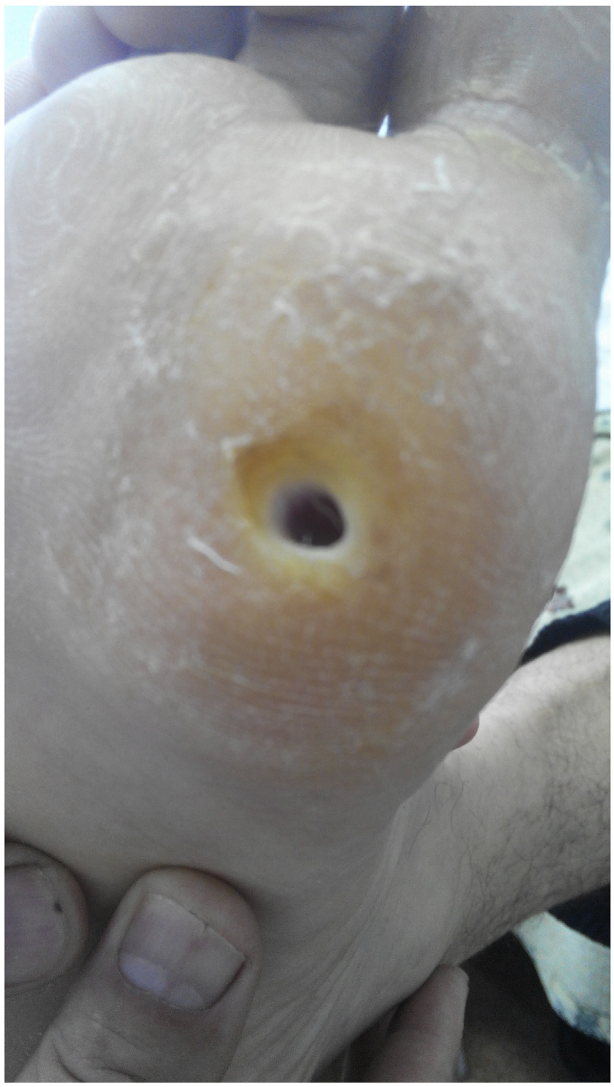

Fig. 2. Dynamics of wound healing-week 1 Dia 0.4–0.5.

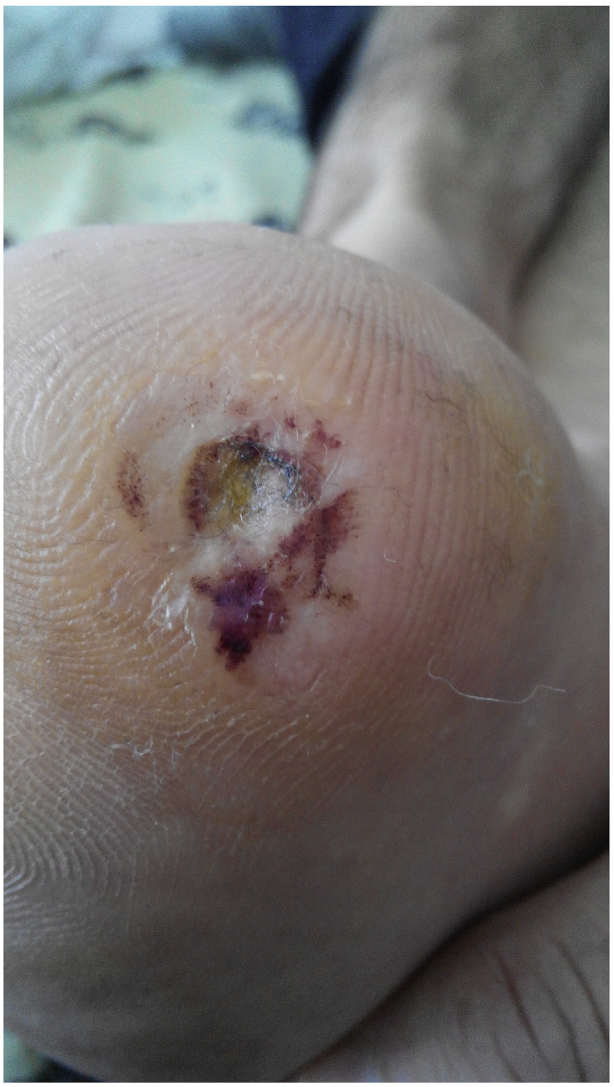

Fig. 3. Dynamics of wound healing-week 2 Dia 0.3–0.4.

1.1. Case description

Patient O is 62 years old, retired, and mostly stays at home. He has had type 2 diabetes for 20 years. During this period the patient developed following diabetes complications as microangiopathy of lower limb, retinopathy, primary cataract and neuropathy. He had also dyslipidemia and hypertension. The patient was a heavy smoker for 20 years. For 15 years patient received oral antidiabetic agents, but in 2011 was initiated with basal insulin therapy due to bad glycemic control. Today patient on basis-bolus insulin therapy with total daily dose of insulin approximately near 54 IU. Concomitant medications included atorvastatin for dyslipidemia and perindopril for hypertension. When he hospitalized to the endocrinology department on June 2014, he had an ulcer on the planter side of the right foot, which had been present for 2 month. As with many of the clinic's patients, Mr O walked barefoot most of the time; however, as a result of his neuropathy, he did not feel the pain that eventually led to the ulcer. He had been treated initially at a local primary healthcare clinic with Trikacide 1500 mg orally per day (Pharmascience Inc), gauze with hypertonic solution (0.45% NaCl), and a retention bandage. Dressings had been changed every other day, although they had caused some trauma to the wound. In spite of treatment for around 2 months the wound was not healing and got infected hence he came to endocrinology department for further management.

Initial local examination. At the first visit to the clinic, the wound measured at diameter 0.8–0.9 cm, with depth of injury 1.7–1.8 cm, appeared to be infected, and was producing scanty and occasional discharge. Areas of necrotic tissue with bed smell were also noted and the periwound skin was with the signs of local inflammation and macerated. Primary debridement was undertaken before commencing the dressing regimen. The criteria for DFU gradation assessment presented at Table 1 [27].

The lab and instrumental data are presented in Table 2. There were no signs of osteomyelitis on Ro. At doppler examination we found no critical stenosis of the main lower limb arteries. After the initial examination the patient was exposed following diagnosis: diabetic foot syndrome, neuropathic, trophic ulcer of right foot, Wagner grade 2.

Fig. 4. Dynamics of wound healing-week 4.

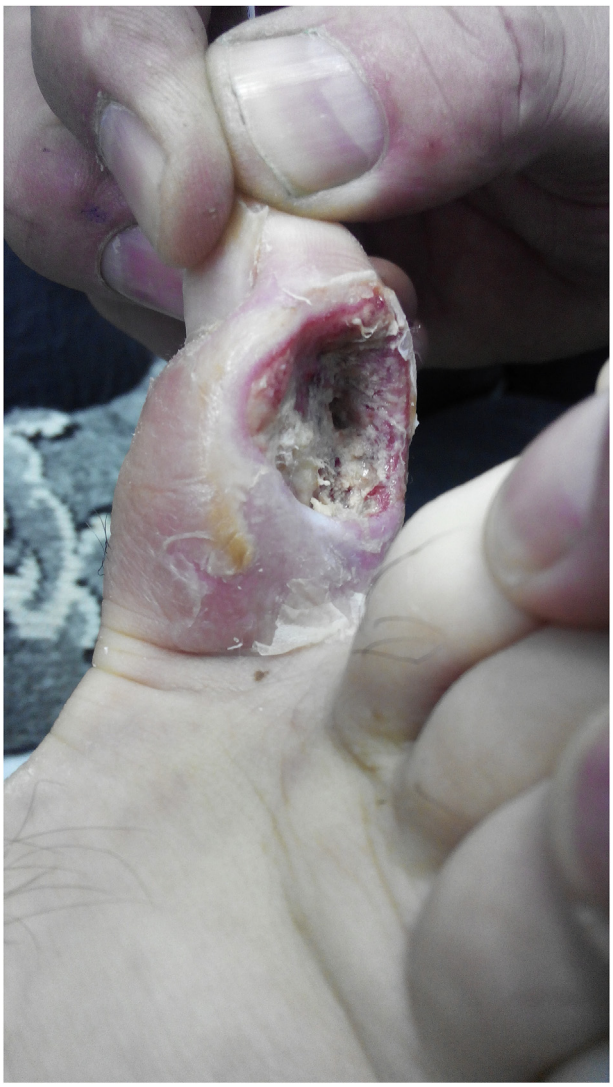

Fig. 5. Dynamics of wound healing-Baseline Dia 2.5–3.0.

Table 4
The dynamics of wound healing.

	Baseline	Week 1	Week 2	Week 4	Week 7	Week 9
Size		++	+	−	+	−
Diameter, мм	2,5–3,0	1,5–2,0	1,5–1,8	1,2–1,3	0,6–0,7	0,2–0,3
Depth, мм	1,5–1,6	0,8–1,0	0,6–0,7	0,2–0,3	0,1–0,2	0
Pain	−	0	0	+	0	0
Edge	++	+	+	0	0	0
Smell	+	+	0	0	0	0
Discharge	+	+/0	+	0	0	0
Surface	++	+	+	0	+	0
	Fig. 5	Fig. 6	Fig. 7	Fig. 8	Fig. 9	Fig. 10

1.2. Treatment plan

- thioctic acid preparations, vitamins B, drugs that enhance metabolism in tissues (Actovegin);
- gauze with hypertonic solution (0.45% NaCl) in the wound channel;
- dermotropic gel based on cerium dioxide nanoparticles in the wound channel 2 times a day (in the morning and evening);
- irrigation with iodine of the wound channel before gel laying;
- antibiotic therapy (lincomycin intramuscular on course for 21 days);
- daily dressings.

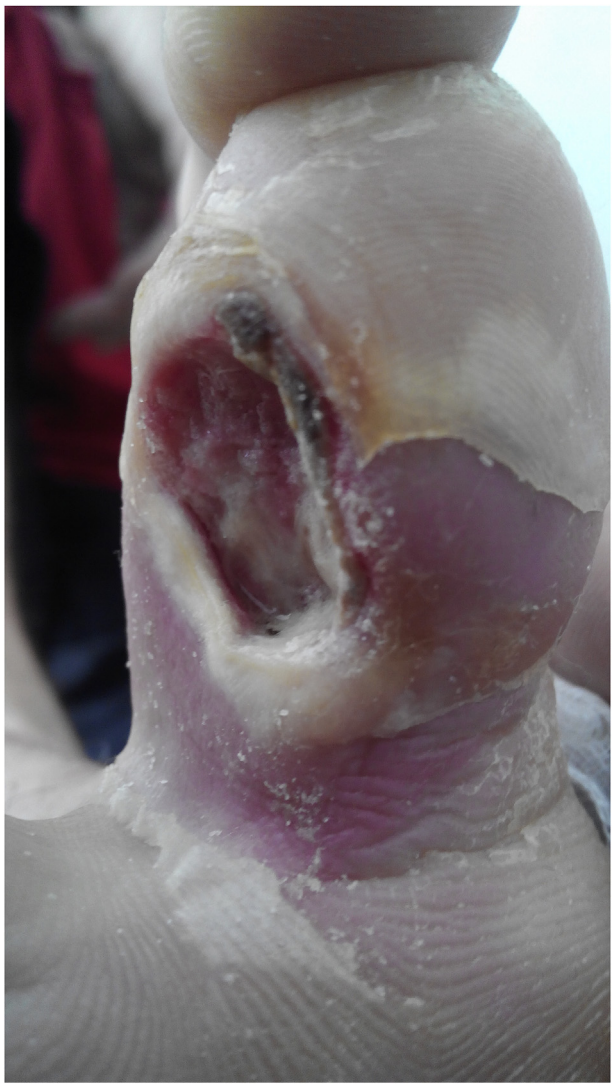

Fig. 6. Dynamics of wound healing-week 1 Dia 1.5–2.0.

Fig. 7. Dynamics of wound healing-week 2 Dia 1.5–1.4.

The description of wound healing dynamics presented at Table 3.

After successful treatment of previous DFU patient was re-hospitalized on October 2014 with recidivism. After initial assessment we diagnosed the trophic DFU of first finger on right foot, Wagner grade 3 and proposed to patient the same treatment strategy. The description of wound healing dynamics presented at Table 4.

2. Discussion

Monafo et al. [28], firstly suggested that cerium due to the antimicrobial properties may have a positive benefit when used on severely burnt patients. Cerium nitrate treatment was well tolerated, altered the burn wound bacterial profile, with a marked decrease in Gram-negative colonisation in general and pseudomonal infection in particular and associated with a nearly 50% reduction in mortality rate. Three further reports [29,30] from the same institution over the following 7 years reiterated similar findings in successively larger groups of patients. In these later series, cerium nitrate with silver sulfadiazine salt (SSD) was the treatment of choice and burn excision was usually delayed until at least the third week. Improvements in bacteriostasis and mortality were similar to those reported initially [14].

Kistler et al. [31] demonstrated in animals that the negative effects attributed to burn toxins could almost completely be prevented by one single early treatment of the burned skin with cerium nitrate. The improvement in survival after Cerium treatment compared to controls (10% vs 74%) was highly significant ($p < 0.0001$) and equated to the survival rates found in burnt animals who underwent immediate burn wound excision and autografting.

Moreover treatment with cerium nitrate obviated many of the detrimental effects of the burn on activated lymphocyte function, with a maintenance of IL-2 receptor activity [32] and prevented the elevation of TNF-alpha levels in the early period after thermal injury [33].

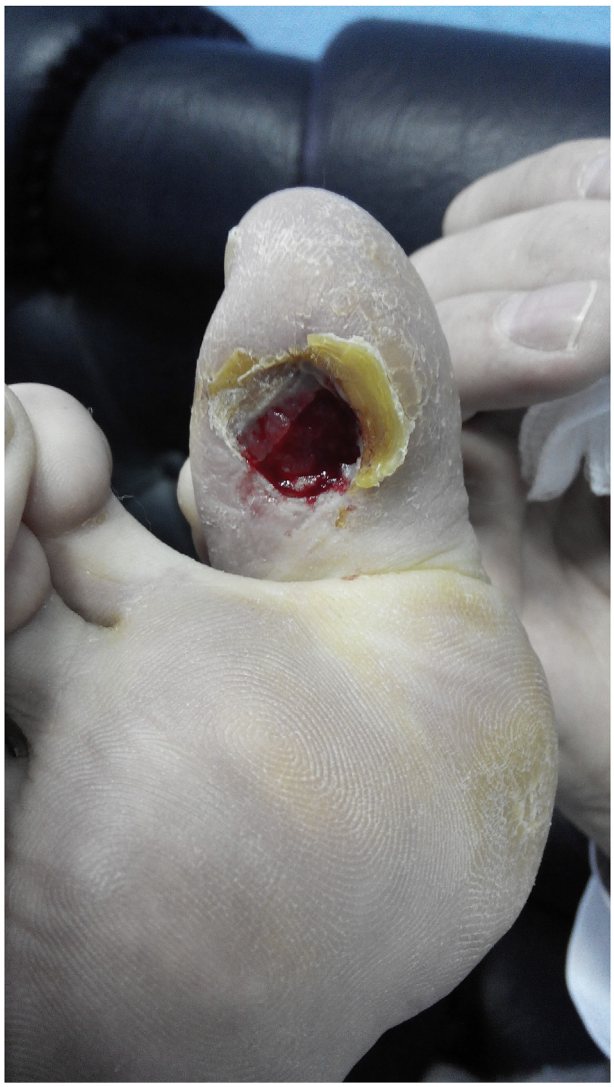

Fig. 8. Dynamics of wound healing-week 4 Dia 1.2–1.3.

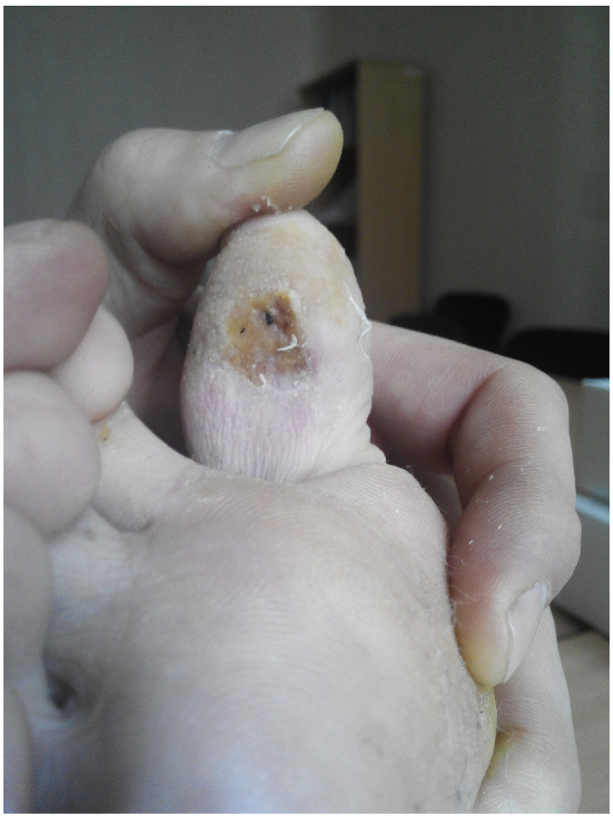

Fig. 9. Dynamics of wound healing-week 7 Dia 0.6–0.7.

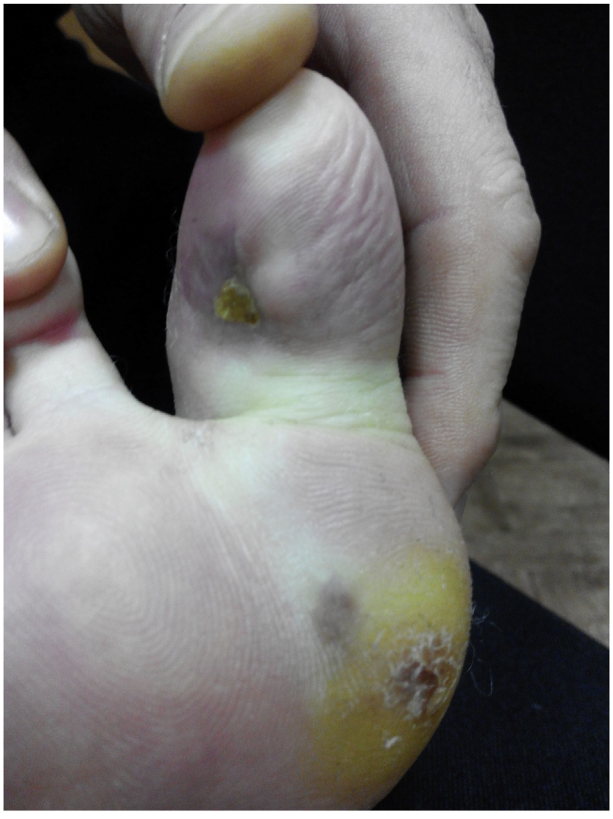

Fig. 10. Dynamics of wound healing-week 9 Dia 0.2–0.3.

Recently Chigurupati et al. reported that topical application of water soluble CNPs accelerates the healing of full-thickness dermal wounds in mice by a mechanism that involves enhancement of the proliferation and migration of fibroblasts, keratinocytes and VECs [34]. The ability of topical application of CNPs to accelerate wound healing in an animal model provides a rationale to develop this technology for use in humans affected by traumatic injury, diabetes and burns.

3. Conclusion

We first described a case report of successful topical treatment of neuropathic diabetic foot ulcers with novel gel containing CNPs. The CNPs penetrated into the wound tissue can reduced oxidative damage and protect regenerative tissue. Due to it bacteriostatic activity and anti-inflammatory properties can prevent secondary infection of the wound, suggesting a therapeutic potential for topical treatment of DFU.

Cure of all diabetic feet in about 1.5–2 months gives us hopes for opening new horizons with CNPs in treatment of DFU as already known to be refractory chronic ulcers. However, more clinical

studies are proposed to be needed to describe a wound healing dynamics in suffering patients.

Conflicts of interest

None to declare.

Acknowledgement

No declare.

References

[1] Boulton AJ, Armstrong DG, Albert SF, Frykberg RG, Hellman R, Kirkman MS, Lavery LA, Lemaster JW, Mills Sr JL, Mueller MJ, Sheehan P, Wukich DK. American Diabetes Association, American Association of Clinical Endocrinologists. Comprehensive foot examination and risk assessment: a report of the task force of the foot care interest group of the ADA, with endorsement by the AACE. Diabetes Care 2008;8:1679–85.
[2] Wu S, Armstrong DG. Risk assessment of the diabetic foot and wound. Int Wound J 2005;2:17–24.
[3] Campbell I. Diabetic foot disease. Br J Diabetes Vasc Dis 2011;11:53–4.
[4] Boulton AJM, Vileikyte L, Ragnarson-Tennvall G, Apelqvist J. The global burden of diabetic foot disease. Lancet 2005;366:1719–24.
[5] Steed DL, Attinger C, Colaizzi T, Crossland M, Franz M, Harkless L, Johnson A, Moosa H, Robson M, Serena T, Sheehan P, Veves A, Wiersme-Bryant L. Guidelines for the treatment of diabetic foot ulcers. Wound Repair Regen 2006;14:680–92.
[6] Singh N, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. J Am Med Assoc 2005;293:217–28.
[7] Schofield CJ, Libby G, Brennan GM, MacAlpine RR, Morris AD, Leese GP. Mortality and hospitalization in patients after amputation: a comparison between patients with and without diabetes. Diabetes Care 2006;29:2252–6.
[8] Broussard CL. Hyperbaric oxygenation and wound healing. J Wound, Ostomy Cont Nurs 2003;30:210–6.
[9] Young M. Ten years of wound care: the truth is out there. Diabet Foot J 2007;10:186–8.
[10] International Working Group on the Diabetic Foot. Practical guidelines on the management and prevention of the diabetic foot (DVD). Amsterdam: IWGDF; 2011.
[11] Thomas S. Atraumatic dressings. World Wide Wounds 2003. Available at: http://www.worldwidewounds.com/2003/january/Thomas/Atraumatic-Dressings.html.
[12] Buchanan RE, Fulmer EI. Physiology and biochemistry of bacteria. II. Effects of environment upon microorganisms. Baltimore: Williams & Wilkins; 1930.
[13] Burkes S, McCleskey CS. The bacteriostatic activity of cerium, lanthanum and thallium. J Bacteriol 1947;54:417–24.
[14] Garner JP, Heppell PS. Cerium nitrate in the management of burns. Burns 2005;5:539–47.
[15] Kobyliak NM, Falalyeyeva TM, Kuryk OG, Beregova TV, Bodnar PM, Zholobak NM. Antioxidative effects of cerium dioxide nanoparticles ameliorate age-related male infertility: optimistic results in rats and the review of clinical clues for integrative concept of men health and fertility. EPMA J 2015;6:12.
[16] Nolan M, Parker SC, Watson GW. Reduction of NO_2 on ceria surfaces. J Phys Chem B 2006;110:2256–62.
[17] Naganuma T, Traversa E. Stability of the Ce3+ valence state in cerium oxide nanoparticle layers. Nanoscale 2012;4:4950–3.
[18] Celardo I, Traversa E, Ghibelli L. Cerium oxide nanoparticles: a promise for applications in therapy. J Exp Therapeut Oncol 2011;9:47–51.
[19] Pirmohamed T, Dowding JM, Singh S, Wasserman B, Heckert E, Karakoti AS, King JE, Seal S, Self WT. Nanoceria exhibit redox state-dependent catalase mimetic activity. Chem Commun 2011;46:2736–8.
[20] Heckert EG, Karakoti AS, Seal S, Self WT. The role of cerium redox state in the SOD mimetic activity of nanoceria. Biomaterials 2008;29:2705–9.
[21] Tarnuzzer RW, Colon J, Patil S, Seal S. Vacancy engineered ceria nanostructures for protection from radiation-induced cellular damage. Nano Lett 2005;5:2573–7.
[22] Hirst SM, Karakoti AS, Tyler RD, Sriranganathan N, Seal S, Reilly CM. Anti-inflammatory properties of cerium oxide nanoparticles. Small 2009;5:2848–56.
[23] Kobyliak N, Abenavoli L, Falalyeyeva T, Virchenko O, Natalia B, Beregova T, Bodnar P, Spivak M. Prevention of NAFLD development in rats with obesity via the improvement of pro/antioxidant state by cerium dioxide nanoparticles. Clujul Med 2016;89:229–35.
[24] Kobyliak N, Virchenko O, Falalyeyeva T, Kondro M, Beregova T, Bodnar P, Shcherbakov O, Bubnov R, Caprnda M, Delev D, Sabo N, Kruzliak P, Rodrigo Opatrilova R, Spivak M. Cerium dioxide nanoparticles possess anti-inflammatory properties in the conditions of the obesity-associated NAFLD in rats. Biomed Pharmacother 2017;90:608–14. https://doi.org/10.1016/j.biopha.2017.03.099.
[25] Golyshkin D, Kobyliak N, Virchenko O, Falalyeyeva T, Beregova T, Ostapchenko L, Caprnda M, Skladany L, Opatrilova R, Rodrigo L, Kruzliak P, Shcherbokov A, Spivak M. Nanocrystalline cerium dioxide efficacy for prophylaxis of erosive and ulcerative lesions in the gastric mucosa of rats induced by stress. Biomed Pharmacother 2016;84:1383–92. https://doi.org/10.1016/j.biopha.2016.10.060.
[26] Das M, Patil S, Bhargava N, Kang JF, Riedel LM, Seal S, Hickman JJ. Auto-catalytic ceria nanoparticles offer neuroprotection to adult rat spinal cord neurons. Biomaterials 2007;28:1918–25.
[27] Dwivedi Amarprakash P. Case study of Leech application in Diabetic foot ulcer. Int J Res Ayurveda Pharm 2012;3:748–51.
[28] Monafo WW, Tandon SN, Ayvazian VH, Tuchsmidt J, Skinner AM, Deitz F. Cerium nitrate: a new topical antiseptic for extensive burns. Surgery 1976;80:465–73.
[29] Fox Jr CL, Monafo Jr WW, Ayvazian VH, Skinner AM, Modak S, Stanford J, Condict C. Topical chemotherapy for burns using cerium salts and silver sulfadiazine. Surg Gynaecol Obstet 1977;144:668–72.
[30] Monafo L. The use of topical cerium nitrate–silver sulfadiazine in major burn injuries. Panminerva Med 1983;25:151–4.
[31] Kistler D, Hafemann B, Schoenenberger GA, Hettich R. Increased survival rates by topical treatment of burns with cerium nitrate. Eur Surg Res 1990;22:283–90.
[32] Eski M, Devici M, Celikoz B, Nisanci M, Turegan M. Treatment with cerium nitrate bathing modulates systemic leukocyte activity following burn injury: an experimental study in rat cremaster muscle flap. Burns 2001;27:739–46.
[33] Deveci M, Eski M, Sengezer M, Kisa U. Effects of cerium nitrate bathing and prompt burn wound excision on IL-6 and TNF alpha levels in burned rats. Burns 2000;26:41–5.
[34] Chigurupati S, Mughal MR, Okun E, Das S, Kumar A, McCaffery M, Seal S, Mattson MP. Effects of cerium oxide nanoparticles on the growth of keratinocytes, fibroblasts and vascular endothelial cells in cutaneous wound healing. Biomaterials 2013;34:2194–201.