Topical Application of Glycerol Increases Penetration Depth of Optical Coherence Tomography in Diagnosis of Basal Cell Carcinoma

Fieke ADAN[1,2], Ellen M. M. OYEN[1,2], Robert J. HOLTACKERS[3], Eva VAN LOO[1,2], Gert-Jan DERMONT[1,2], Nicole W. J. KELLENERS-SMEETS[1,2], Patty J. NELEMANS[4] and Klara MOSTERD[1,2]

[1]Department of Dermatology, [3]Department of Radiology & Nuclear Medicine, Maastricht University Medical Centre, [2]GROW Research Institute for Oncology and Developmental Biology and [4]Department of Epidemiology, Maastricht University, Maastricht, The Netherlands

Optical coherence tomography is a non-invasive imaging technique that enables high-resolution *in vivo* imaging of skin. Although optical coherence tomography is promising for diagnosing basal cell carcinoma, its limited penetration depth may impede basal cell carcinoma subtyping. This study evaluated whether topical application of glycerol can increase penetration depth and improve the image quality and visibility of characteristic features of basal cell carcinoma. A total of 61 patients with a total of 72 basal cell carcinomas were included. Optical coherence tomography scans were obtained before and after application of an 85% glycerol solution. The mean penetration depth of each optical coherence tomography scan was acquired by automatically tracing both skin surface and the point of signal loss using a custom-made MATLAB program. Mean ± standard deviation penetration depth increased from 883 ± 108 to 904 ± 88 µm before and after glycerol application, respectively ($p = 0.005$). Topical application of glycerol leads to a significant 2.4% increase in penetration depth. However, no significant differences in image quality and visibility of basal cell carcinoma features were found.

Key words: optical coherence tomography; basal cell carcinoma; optical clearing agents.

Acccepted Apr 26, 2021; Epub ahead of print Apr 27, 2021

Acta Derm Venereol 2021; 101: adv00474.

Corr: Fieke Adan, Department of Dermatology, Maastricht University Medical Centre, P. Debyelaan 25, NL-6229 HX, Maastricht, The Netherlands. E-mail: fieke.adan@mumc.nl

The incidence of non-melanoma skin cancer (NMSC) is increasing globally, with basal cell carcinoma (BCC) being the most prevalent skin cancer diagnosed among the Caucasian population (1). Histopathological examination of a punch biopsy remains the gold standard for confirming BCC diagnosis and subtype (2, 3). However, a punch biopsy is a minor invasive procedure.

Optical coherence tomography (OCT) has emerged as a promising non-invasive imaging technique for the diagnosis of BCC, showing improved specificity and sensitivity when used in addition to clinical examination and dermoscopy (3–6). OCT uses the reflection of an optical beam to acquire real-time cross-sectional images of the skin with a < 7.5 µm lateral and < 5 µm axial optical resolution, and a penetration depth of approximately 1–1.5 mm.

SIGNIFICANCE

Optical coherence tomography is a promising non-invasive diagnostic technique for the diagnosis of basal cell carcinoma; however, its limited penetration depth may impede basal cell carcinoma subtyping. By reducing light scattering in optical coherence tomography scans, using optical clearing agents, such a glycerol, the penetration depth may be enhanced. This study included 61 patients with a total of 72 basal cell carcinomas. Optical coherence tomography scans were obtained before and after application of an 85% glycerol solution. Mean penetration depth increased significantly, by 2.4%, after application of glycerol.

Based on the optical reflections, the epidermis, dermis, and skin appendages can be distinguished (6, 7). However, as the mean tumour depth of aggressive BCC subtypes, including infiltrative and micronodular BCC, is estimated at approximately 1.5 mm, the penetration depth may be insufficient to detect deeper located and smaller BCC tumour nests (8).

By reducing light scattering in OCT scans, the penetration depth may be enhanced. Light scattering occurs mainly at the tissue interfaces whose refractive indices mismatch, such as the surface of skin and the dermal-epidermal border. In pursuance of enhancing OCT image quality and penetration depth, hyperosmotic chemical agents, called optical clearing agents (OCAs), have been applied to the skin to match refractive indices. These OCAs reduce light scattering and thereby enhance optical penetration depth (9, 10). Glycerol, a hydrophilic trihydroxy alcoholic substance, has been used as OCA in multiple studies, demonstrating increased penetration depth and enhanced contrast in OCT diagnostics (9–14). However, the reported increase in penetration depth has not yet been quantified.

The aims of this study were to evaluate whether topical application of glycerol solution on BCCs improves optical penetration depth. In addition, the effect of glycerol application on image quality and visibility of characteristic BCC features was evaluated (15).

MATERIALS AND METHODS

Patients, aged 18 years or older, visiting the department of dermatology of the Maastricht University Medical Centre+ (MUMC+)

with 1 or more histopathologically confirmed BCCs were included between January and May 2019. The study was approved by the local ethics committee (METC 16-4-197) and was conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from all patients prior to inclusion.

Optical coherence tomography imaging

OCT imaging of the BCC(s) was performed both before and immediately after topical application of glycerol 85% (0.01 ml) solution on the skin lesion. All OCT scans were acquired by a single physician using a commercially available OCT device (VivoSight; Michelson Diagnostics Ltd, Maidstone, UK) equipped with a 6-mm probe (axial resolution 15 µm). Prior to OCT imaging, a medical photograph was taken of each lesion.

Image analysis

For all OCT images the mean penetration depth was assessed using a custom-made MATLAB (version 2018b; The Mathworks, Natick, MA, USA) script. This program automatically traced the skin surface and the point of signal loss for each location in the image based on the (differences in) signal intensity, represented by blue and red lines in the OCT image, respectively (**Fig. 1**). Penetration depth was defined as the mean distance between these 2 lines. Subsequently, all OCT images were presented in random order to 3 observers who were blinded to any patient data and did not know whether the OCT image was taken before or after application of glycerol: 1 dermatologist with extensive OCT imaging experience (EvL) and 2 dermatology residents with moderate OCT imaging experience (EO and GD).

Observers scored the overall image quality (determined by the noise level and shadows casted by keratosis and/or crusts/ulcerations), and visibility of the most common features of BCC (as identified previously by Hussain et al. (15)). Both parameters were scored separately using a 4-point Likert-scale (1: low, 2: medium, 3: high, and 4: very high). Since lower image quality has been reported for BCCs presenting with keratosis and with crusts and/or ulcerations, lesions with these features were classified into 2 subgroups based on clinical presentation (16).

Statistical analysis

The sample size calculation was based on the mean penetration depth, the primary outcome measure. An increase in mean penetration depth after glycerol application of half the standard deviation (SD) of the difference (effect size 0.5) was considered as minimally clinically relevant. To enable detection of such a difference between the 2 conditions (before and after topical application of glycerol) with a power of 80% and 2-sided alpha of 5%, 64 BCCs were required. To account for a 10% drop-out rate 72 BCCs were included.

Results are expressed as mean ±SD or as percentage, unless otherwise specified. Differences in mean penetration depth before and after topical application of glycerol were evaluated using either a paired-samples Student's *t*-test (in case of normally distributed data) or a non-parametric Wilcoxon signed-rank test (in case of non-normally distributed data). Normality of the data was evaluated using the Shapiro–Wilk test. Differences in image quality and visibility of characteristic BCC features before and after topical glycerol application were evaluated using the McNemar's test for paired proportions. The proportions of OCT scans with higher

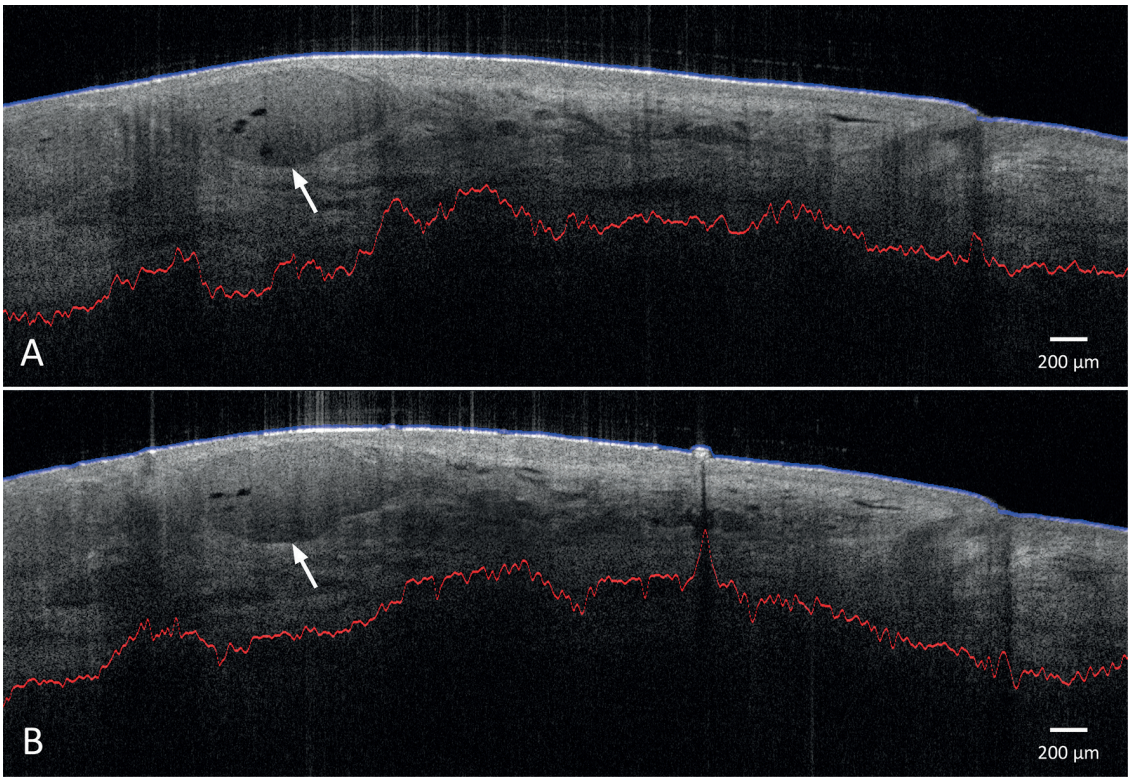

Fig. 1. Optical coherence tomography (OCT) images of the same basal cell carcinoma (BCC) acquired before (image A) and after (image B) application of glycerol. A custom-made MATLAB program was used to automatically analyse the images: the *blue line* traces the skin surface, while the *red line* traces the point of signal loss. The distance between these 2 lines was calculated at all positions to obtain mean penetration depth. A signal-poor ovoid nest (corresponding to a basaloid cell nest) is indicated by the *white arrow*. At approximately 1-mm depth the signal intensity drastically decreases, which might make it difficult to identify features of BCC in deeper skin layers. Note that images (A) and (B) differ slightly in image position as the OCT imaging probe was removed for application of glycerol.

scores after application of glycerol (improved outcome) were compared with percentages with lower scores after application of glycerol (worsened outcome). Separate analyses were performed for the 2 subgroups of BCCs presenting with keratosis, or crusts and/or ulcerations and for superficial BCCs. All statistical analyses were performed using SPSS Statistics 25 (International Business Machines (IBM), Armonk, NY, USA).

Two-sided p-values <0.05 were considered statistically significant.

RESULTS

Sixty-one patients (35 male, median age 70 years, age range 44–95 years) with a total of 72 BCCs were included. Baseline characteristics of the study sample are summarized in **Table I**. OCT imaging was performed successfully before and after topical application of glycerol in all patients.

The mean ± SD penetration depth increased significantly after topical application of glycerol (883 ± 108 vs 904 ± 88 µm, $p=0.005$). The 21-µm difference represented an increase by 0.34 SD of the difference corresponding with an effect size of 0.34.

The numbers and proportions of BCCs with improved and reduced scores on the 4-point Likert scale, with respect to overall image quality and visibility of characteristic BCC features after glycerol application, are shown in **Table II**. Regarding overall image quality after glycerol application, no significant improvement was found for observers 1 and 3. For observer 2, the proportions with improved scores were substantially higher than the proportions with reduced scores, but only statistically significant for BCC with crusts and/or ulcerations ($p=0.04$). Regarding the visibility of BCC features, there was a trend toward improved scores for observers 1 and 2, but the results were not statistically significant. For observer 1, the visibility of BCC features for superficial BCCs significantly decreased ($p=0.01$).

Table I. Baseline characteristics of the study sample (61 patients with a total of 72 basal cell carcinomas (BCCs))

Characteristics	
Sex (male), *n* (%)	35 (57.4)
Age, years, median (range)	70 (44–95)
Number of lesions per patient, *n* (%)	
1	53 (86.9)
2	6 (9.8)
3	1 (0.02)
4	1 (0.02)
Lesion location n (%)	
Head and neck region	23 (31.9)
Upper chest	6 (8.3)
Back/abdomen	27 (37.5)
Extremities	16 (22.2)
BCC subtype	
Superficial	26 (36.1)
Nodular	28 (38.9)
Mixed nodular/superficial	13 (18.1)
Infiltrating/morpheaform	2 (2.8)
Mixed nodular/morpheaform	1 (1.4)
Mixed superficial/micronodular	1 (1.4)
Mixed nodular/micronodular	1 (1.4)

Table II. Proportions of improved score, equal score, and reduced score for overall image quality and visibility of basal cell carcinoma (BCC) features after topical glycerol application for all 3 observers

Outcome	Observer	Improved score[a] % (*n*)	Equal score % (*n*)	Reduced score % (*n*)	*p*-value
All ($n=72$)					
Overall image	1	26.4 (19)	41.7 (30)	31.9 (23)	0.79
quality after glycerol	2	38.9 (28)	45.8 (33)	15.3 (11)	0.08
application	3	22.2 (16)	47.2 (34)	30.6 (22)	0.20
Visibility of BCC	1	25.0 (18)	56.9 (41)	18.1 (13)	0.70
features after glycerol	2	38.9 (28)	31.9 (23)	29.2 (21)	0.49
application	3	18.1 (13)	56.9 (41)	25.0 (18)	0.06
Keratosis ($n=42$)					
Overall image	1	28.6 (12)	45.2 (19)	26.2 (11)	0.84
quality after glycerol	2	42.9 (18)	45.2 (19)	11.9 (5)	0.07
application	3	19.0 (8)	57.1 (24)	23.8 (10)	0.64
Visibility of BCC	1	28.6 (12)	52.4 (22)	19.0 (8)	0.22
features after glycerol	2	42.9 (18)	31.0 (13)	26.2 (11)	0.51
application	3	19.0 (8)	54.8 (23)	26.2 (11)	0.37
Crust/ulceration ($n=19$)					
Overall image	1	26.3 (5)	47.4 (9)	26.3 (5)	0.48
quality after glycerol	2	52.6 (10)	31.6 (6)	15.8 (3)	0.04
application	3	26.3 (5)	52.6 (10)	21.1 (4)	0.56
Visibility of BCC	1	26.3 (5)	57.9 (11)	15.8 (3)	0.77
features after glycerol	2	47.4 (9)	36.8 (7)	15.8 (3)	0.16
application	3	21.1 (4)	57.9 (11)	21.1 (4)	0.76
Superficial BCC ($n=26$)					
Overall image	1	38.5 (10)	19.2 (5)	42.3 (11)	0.15
quality after glycerol	2	38.5 (10)	46.2 (12)	15.4 (4)	0.46
application	3	15.4 (4)	65.4 (17)	19.2 (5)	0.74
Visibility of BCC	1	27.0 (7)	15.4 (4)	57.7 (15)	0.01
features after glycerol	2	46.2 (12)	15.4 (4)	38.5 (10)	0.88
application	3	19.2 (5)	61.5 (16)	19.2 (5)	0.78

Results are presented as % (*n*) with corresponding *p*-values (McNemar's test).
[a]An improved score is defined as an increase on the 4-point Likert scale.
1: EvL, 2: EO, and 3: GD.

DISCUSSION

The main objective of this study was to evaluate whether topical application of glycerol increases the optical penetration depth, which may aid the detection of deeper located BCC tumour nests. This study demonstrates that application of glycerol increases penetration depth from 883 to 904 µm, corresponding to an effect size of 0.34. This limited increase, however, may not be sufficient to detect aggressive BCC tumour nests, which can reach an estimated mean depth of 1,500 µm.

The observed penetration depth was remarkably lower than expected, as a systematic review reports a mean penetration depth of 1.2–2 mm with the same OCT device as that used in the current study (17). We found that beyond 1-mm depth the signal intensity decreases drastically (Fig. 1), even after application of glycerol. Reported penetration depths of other devices vary from 1–1.6 mm (Thorlabs, Newton, NJ, USA), 1.3 mm (Risø National Laboratory, Roskilde, Denmark) and 2.0–2.5 mm (an OCT device developed at the Technical University of Denmark) (17).

Despite the increase in penetration depth, no improvement in image quality and visibility of BCC features was found. This may be explained by the fact that resolution, more than penetration depth, determines image quality and how well BCC features can be distinguished from surrounding tissue.

Although OCAs may be useful for OCT imaging, Welzel et al. (14) concluded that topical treatment of the skin prior to OCT imaging is not imperative, but gives a non-specific increase in optical penetration depth due to the lower surface reflectivity. They found that a decrease in the light attenuation coefficient implies an increase in optical penetration depth, although this increase was not exactly quantified. Different solutions, including glycerol, ultrasonic gel, urea, petrolatum and paraffin oil, were tested on healthy skin of the fingertips in 15 patients. OCT images were obtained directly after application and compared with the untreated fingertips of the other hand (14). All investigated solutions resulted in a comparable decrease in surface reflectivity and increase in optical penetration depth. Wang et al. (10) used a combined liquid paraffin and glycerol mixture to reduce light scattering in tissue and achieve more optical penetration depth. Eight OCT images of human fingers were obtained at 0–40 min after application, with a 5-min interval between each image. The time to reach the optimal optical clearing effect, defined as an OCT image with enhanced contrast, was around 10–30 min after application of a mixture with 70% glycerol concentration. The authors concluded that applying the liquid paraffin and glycerol mixture led to an OCT scan with enhanced contrast and assumed that this indicated an increase in optical penetration depth, although this increase was not exactly quantified.

Even though the above-mentioned studies report an increase in optical penetration depth and enhanced contrast after glycerol application, it was not reported whether these findings led to improved image quality and visibility of BCC features in OCT images. Wang et al. (10) observed enhanced contrast after application of glycerol, but in the current study an improvement in image quality and visibility of BCC features was not observed.

In conclusion, topical application of glycerol increases the optical penetration depth in OCT imaging of skin lesions suspected for BCC. However, this limited increase may not be clinically relevant. No significant differences were found in image quality and visibility of BCC features after topical application of glycerol.

The authors have no conflicts of interest to declare.

REFERENCES

1. Flohil SC, de Vries E, Neumann HA, Coebergh JW, Nijsten T. Incidence, prevalence and future trends of primary basal cell carcinoma in the Netherlands. Acta Derm Venereol 2011; 91: 24–30.
2. NVDV. Multi-disciplinary evidence-based guideline basal cel carcinoma. [accessed december 2020] Available from: www.oncoline.nl. Utrecht, 2015.
3. Peris K, Fargnoli MC, Garbe C, Kaufmann R, Bastholt L, Seguin NB, et al. Diagnosis and treatment of basal cell carcinoma: European consensus-based interdisciplinary guidelines. Eur J Cancer 2019; 118: 10–34.
4. Ferrante di Ruffano L, Dinnes J, Deeks JJ, Chuchu N, Bayliss SE, Davenport C, et al. Optical coherence tomography for diagnosing skin cancer in adults. Cochrane Database Syst Rev 2018; 12: CD013189.
5. Sinx KAE, van Loo E, Tonk EHJ, Kelleners-Smeets NWJ, Winnepenninckx VJL, Nelemans PJ, et al. Optical coherence tomography for noninvasive diagnosis and subtyping of basal cell carcinoma: a prospective cohort study. J Invest Dermatol 2020; 140: 1962–1967.
6. Ulrich M, von Braunmuehl T, Kurzen H, Dirschka T, Kellner C, Sattler E, et al. The sensitivity and specificity of optical coherence tomography for the assisted diagnosis of nonpigmented basal cell carcinoma: an observational study. Br J Dermatol 2015; 173: 428–435.
7. Mogensen M, Joergensen TM, Nurnberg BM, Morsy HA, Thomsen JB, Thrane L, et al. Assessment of optical coherence tomography imaging in the diagnosis of non-melanoma skin cancer and benign lesions versus normal skin: observerblinded evaluation by dermatologists and pathologists. Dermatol Surg 2009; 35: 965–972.
8. Pyne JH, Myint E, Barr EM, Clark SP, Hou R. Basal cell carcinoma: variation in invasion depth by subtype, sex, and anatomic site in 4,565 cases. Dermatol Pract Concept 2018; 8: 314–319.
9. Shan H, Liang Y, Wang J, Li Y. Study on application of optical clearing technique in skin diseases. J Biomed Opt 2012; 17: 115003.
10. Wang B, Wang HW, Guo H, Anderson E, Tang Q, Wu T, et al. Optical coherence tomography and computer-aided diagnosis of a murine model of chronic kidney disease. J Biomed Opt 2017; 22: 1–11.
11. Liew YM, McLaughlin RA, Wood FM, Sampson DD. Reduction of image artifacts in three-dimensional optical coherence tomography of skin in vivo. J Biomed Opt 2011; 16: 116018.
12. Tycho AAP, Thrane L, Jemec GBE. Optical coherence tomography in dermatology. In: Serup J, Jemec GBE, Grove GL, editors. Handbook of non-invasive methods and the Skin, 2nd edn**.** Boca Raton, Florida: Taylor and Francis Group; 2006, p. 257–266.
13. Fluhr JW, Darlenski R, Surber C. Glycerol and the skin: holistic approach to its origin and functions. Br J Dermatol 2008; 159: 23–34.
14. Welzel J, Reinhardt C, Lankenau E, Winter C, Wolff HH. Changes in function and morphology of normal human skin: evaluation using optical coherence tomography. Br J Dermatol 2004; 150: 220–225.
15. Hussain AA, Themstrup L, Jemec GB. Optical coherence tomography in the diagnosis of basal cell carcinoma. Arch Dermatol Res 2015; 307: 1–10.
16. Holmes J, von Braunmuhl T, Berking C, Sattler E, Ulrich M, Reinhold U, et al. Optical coherence tomography of basal cell carcinoma: influence of location, subtype, observer variability and image quality on diagnostic performance. Br J Dermatol 2018; 178: 1102–1110.
17. Cheng HM, Guitera P. Systematic review of optical coherence tomography usage in the diagnosis and management of basal cell carcinoma. Br J Dermatol 2015; 173: 1371–1380.