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Carbon Dioxide Laser Ablation of Basal Cell Carcinoma with Visual Guidance by Reflectance Confocal Microscopy: A Proof of Principle Pilot Study

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Abstract

Background—Laser ablation is an alternative, non-surgical treatment modality for low-risk basal cell carcinoma (BCC); however, lack of confirmative tumour destruction or residual tumour presence has been a limiting factor to adoption. Reflectance confocal microscopy (RCM) provides non-invasive, cellular-level resolution imaging of the skin and is capable of identifying tumour.

Objective—To evaluate the use of RCM to guide carbon dioxide (CO₂) laser ablation of BCC, confirm destruction, and correlate findings with histology.

Methods—RCM was used pre-ablation to evaluate for features of BCC. Ablation was performed with a CO₂ laser, and the response rapidly assessed using handheld RCM to evaluate for residual tumour. Confirmative pathology was used to verify confocal imaging.

Results—RCM imaging identified tumour pre-ablation with features not identified on normal, surrounding skin. Post-ablation, RCM documented complete removal of tumour in six cases and residual tumour in two. Histologic examination identified the ablated area and confirmed clearance of tumour in the six aforementioned cases and corroborated confocal findings for residual tumour in the other two cases.

Conclusions—We report successful treatment of superficial and nodular BCC using CO₂ laser ablation augmented by RCM imaging for pre-ablation guidance and verification of tumour removal post-ablation. Akin to complete circumferential and deep margin control techniques, using RCM helps to map peripheral and deep BCC margins to hone in on areas exhibiting persistent tumour after ablation. CO₂ laser ablation visually guided by RCM can help circumvent previously cited limiting factors of laser ablation for tumour destruction by providing cellular-level resolution imaging of tumour and margin assessment in between each laser pass and post-ablation.

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Introduction

Nearly 5 million people are treated annually for skin cancer in the United States,¹ with basal cell carcinoma (BCC) accounting for approximately 80% of all cases^{2,3} and its incidence is increasing worldwide.² BCC tumours may have high morbidity with functional and cosmetic impairment and harbours significant healthcare costs. The treatment modality depends on the size, location, and histologic subtype of BCC as well as individual patient comorbidities and preference.⁴ Surgical excision is the gold standard for treatment of high risk BCCs, but may lead to excess tissue removal, scarring, and can be challenging in certain locations.⁵ Lower risk BCCs include superficial and nodular subtypes located on the trunk and extremities. For lower risk lesions, a number of destructive modalities exist, including electrodesiccation and curettage or cryosurgery. Laser ablation may be an alternative therapy for treating superficial and early nodular basal cell carcinomas and a number of different lasers, including carbon dioxide (CO₂), pulsed dye, erbium:yttrium aluminum garnet (Er:YAG), and neodymium:yttrium aluminum garnet (Nd:YAG) have been explored for treatment of BCCs.⁶⁻⁹

CO₂ lasers emit long wave infrared light at a wavelength of 10,600nm that is strongly absorbed by water.¹⁰ By selectively targeting water as the principle chromophore in the skin, the CO₂ laser can coagulate and destroy tissue. Studies have documented successful treatment of BCCs with good cosmetic outcome using CO₂ lasers,¹⁰⁻¹⁴ however a limitation is the lack of confirmation of complete tumour destruction.

Reflectance confocal microscopy (RCM) is a non-invasive imaging modality providing real-time imaging of the skin with cellular-level resolution. RCM has been shown to detect features of BCC in vivo and ex vivo.^{15,16} The ability to immediately assess tissue with RCM may aid the controlled tissue removal of BCCs with CO₂ laser ablation by providing real-time feedback in between passes.

Herein, we describe a proof of principle, pilot study where we combine confocal imaging with CO₂ laser ablation, allowing for confirmation of diagnosis, image-guided ablation of the tumour between passes, and evaluation of clearance in one treatment.

Methods

Patients presenting between June 2014 and January 2015 with clinically visible, shave biopsy-proven superficial or nodular BCCs (one lesion with both a nodular and infiltrative component), located in low risk areas, were recruited for participation in this prospective, proof of principle, non-randomized study under IRB approval. All patients agreed to undergo initial RCM of the lesion, followed by CO₂ laser ablation of the delineated tumour, re-imaging with RCM to detect the absence or presence of tumour between laser passes, and lastly definitive pathological confirmation. For pathological confirmation and correlation, the lesion was excised using the Mohs Micrographic surgery (MMS) technique to ensure complete circumferential and deep margin evaluation. After the peripheral and deep margin was deemed clear via en face sectioning, the tissue block was also processed in a traditional breadloaf fashion to correlate pathology with laser ablation. Patients were excluded if they

were pregnant or had taken isotretinoin within the last year. The primary goal was to demonstrate the feasibility of using RCM to visually guide laser ablation of BCCs before, in-between, and after laser ablation.

Pre-treatment RCM Imaging

Pre-operatively, the clinical borders of the lesion were demarcated. An 8mm by 8mm area centered within the tumour was selected for RCM imaging and ablation. Surrounding tumour served as a control when the lesion was greater in size. If the lesion was smaller than 8mm x 8mm, half of the lesion was selected for ablation with the remaining portion serving as a control. The location of the imaged and ablated area was defined and recorded by demarcating four quadrants using 12, 3, 6, and 9 o'clock reference points denoted by surgical hash marks. The same reference marks were used for MMS for comparison and registration of the confocal imaging with histology. Initial mapping with RCM was performed using the scanning Vivascope 1500 (Caliber I.D. [formerly Lucid Inc], Rochester, NY; field of view 0.5mm x 0.5mm). The Vivascope 1500 is capable of scanning the skin capturing a grid of 256 images (16 by 16) to produce a confocal mosaic (Vivablock) over an area of 8mm by 8mm (Fig. 1). This was performed at the epidermis, dermoepidermal junction, and reachable deep dermis, allowing for designation of the lateral and deep borders of the tumour. Additionally, stacks of images were taken in the z-plane to better define tumour depth up to 150–200 μ m. Imaging the lesion in both the horizontal and vertical axes supplied a 3-D approximation of tumour margins.

CO2 Laser Ablation

Local anesthetic was injected and ablation was performed with the CO2 laser (Lumenis Ultrapulse 5000C; San Jose, CA, USA). All patients received one treatment performed by the same physician (AMR). Laser treatment was performed using the following clinically-relevant parameters based on ex vivo work of this method on excised BCC tissue:¹⁷ wavelength 10,600nm, pulse duration 750 μ sec, fluence of 7.5J/cm², square pattern varying in size from 2 mm to 9mm, and density of 30% for uniform ablation. The number of passes was dictated by the presence or absence of tumour on RCM imaging between each pass. If tumour was present another laser pass was performed in that area. The resultant white char was wiped clean with sterile water and dried in-between passes with a cotton swab.

RCM Imaging In-Between Passes and Post Ablation

In-between each laser pass (up to 3 passes), the ablated area was re-imaged with RCM to determine tumour presence or confirm tumour destruction. To enhance the contrast of residual BCC on confocal microscopy, 35% aluminum chloride was topically applied post-ablation using four swabs for 15 seconds each, totaling 1 minute.¹⁸ Topical aluminum chloride was not applied prior to the first round of ablation to enhance RCM imaging due to its limited penetration of intact skin. Sterile gel was applied for post-ablation imaging with the smaller, handheld Vivascope 3000 (Caliber I.D. [formerly Lucid Inc], Rochester, NY; field of view 0.75mm x 0.75mm), utilizing caps sterilized with gas sterilization. The handheld Vivascope 3000 was used to rapidly assess the area of ablation, and real-time videos were captured moving in a clockwise direction of each quadrant (Figs 2–3). Videos were then rendered into confocal strip mosaics based on previous work by our group.¹⁹

Stacks of images were collected in the z-plane within and adjacent to the area of ablation from the most superficial keratinocytes down to the point at which tumour features were not discernable, allowing for an estimation of the depth of ablation. Areas suspicious for residual tumour were examined with additional stacks of images. The ablation and imaging procedure was repeated for 2–3 passes for each lesion. In the case of persistent residual tumour identified on imaging after repeated passes of the laser, the quadrant location within the lesion was noted for histologic correlation.

Histologic examination

The entire lesion was excised via the MMS technique for all cases as standard of care using an initial 3 millimetre margin. That tissue was processed en face for margin (perimeter and deep margin) examination and histopathologic correlation (Figs 2–3). The frozen tissue was then thawed and re-embedded for vertical frozen sections tip-to-tip (traditional breadloafing) for further histologic inspection. The same 12 o'clock reference point used in imaging and MMS was used for bread loafing the tissue block to ensure registration of the reference points. One section was selected per 100 microns for hematoxylin and eosin staining to validate the destruction of tumour with laser ablation and to register with the confocal findings. Depth of ablation was estimated by measuring the distance from the adjacent, intact epidermis down to the level of ablation at two sites flanking the area of ablation and averaging these values for comparison with the estimated depths seen on confocal microscopy.

Results

Seven patients with eight lesions were treated (Table 1). Initial RCM imaging confirmed presence of BCC in all 8 lesions. The shave biopsy did not impair our ability to detect tumour with RCM, and features of fibrosis were seen in the dermal layer. BCC tumour characteristics included dense, nodular areas with surrounding cleft-like spaces, polarization of elongated basaloid nuclei, and increased tumour vasculature as assessed by real-time blood vessel trafficking. The BCC features seen on confocal were not identified on normal, surrounding skin, used as control.

All patients tolerated the procedure well with no adverse outcomes. The hemostasis provided by the CO₂ laser created a virtually bloodless operating field such that RCM imaging quality was not impaired. RCM imaging within the ablated region after 2–3 passes of the laser identified complete removal of tumour in six cases and showed persistent signs of residual tumour in two cases (Table 1). The relative location of the residual tumour was identified with confocal imaging for correlation with histology. Stacks of images captured in the z-plane estimated the depth of ablation, which ranged from 80µm to 120µm (average estimated depth 94µm).

All tumours were removed with clear margins after one stage of MMS, done for histological confirmation in this study. Complete tissue block histologic examination identified areas ablated by the laser and confirmed the complete clearance of tumour in the six aforementioned cases within the area ablated. The histology also correlated with confocal findings for the persistence of tumour in the two cases after ablation with accurate

registration between the area of residual tumour identified on confocal microscopy and the area of persistent tumour on histology. The estimated depths of ablation as measured on histology ranged from 72.5µm to 150µm (average depth 102µm).

Discussion

This initial pilot, proof of principle study demonstrates the feasibility of treating superficial and early nodular BCCs with a CO₂ laser, guided by real-time confocal microscopy. While destruction of BCCs has been documented with ablative lasers, the lack of histological confirmation of tumour destruction has made this method less favorable. Using RCM to visually detect BCC and guide CO₂ ablation in our study is novel and represents a way to non-invasively confirm tumour presence or clearance. RCM can help refine and hone the CO₂ laser ablation for precise destruction of BCCs without excessive fluence or passes. We were able to definitively correlate the ablated field on confocal with histology confirming that the areas seen on confocal were indeed ablated. The choice to only ablate a representative section of larger BCCs provides an internal control on histology as the zone of laser ablation versus residual tumour could easily be demarcated under both RCM and histology (Fig. 2–3).

The choice of CO₂ laser is based on the ability of this wavelength to both effectively ablate tissue down to a depth that is needed and simultaneously coagulate tissue. The CO₂ laser has an absorption coefficient of 800cm⁻¹ and thermal coagulation zone between 80µm and 150µm. This thermal coagulation zone is larger compared to other ablative wavelengths thus providing increased hemostasis as well as a larger zone of destruction with fewer passes and fluence needed.

In this study, 6 of the 8 BCCs were cleared within the area of ablation (confirmed on both RCM and histology) using 2–3 passes of the CO₂ laser. Density and fluence were kept constant to determine the efficacy of these parameters. Increasing the fluence and density would increase our depth of ablation and coverage, respectively, and may be appropriate for more nodular or infiltrative tumours. Our pre-ablation RCM confirmed tumour in all cases, highlighting previously reported BCC features such as polarized nuclei and tumour nests with clefting.^{7,20} A rapid, preoperative evaluation using RCM to categorize BCC subtype is important when selecting a treatment modality. While specific RCM criteria have been shown to assist in correct BCC classification, there may exist the potential risk to underestimate or overestimate BCC subtype (such as morpheaform) on account of its peculiar morphology and confocal appearance.²¹ One novel application of RCM after laser ablation is the ability to visualize layers of the skin in the z-plane and estimate the depth of ablation. We were able to discern ablated tissue down to 150µm, and were able to image deeper in the center of the ablated wound by increasing pressure on the scope. The ability to measure the depth of the tumour and ablation will assist in adjusting the fluence of the laser accordingly to achieve that level of penetration in the future. Our estimated depths of ablation seen with confocal microscopy (range 80–120µm; average 94µm) were very similar to the depths of ablation as assessed on histology (range 72.5–150µm; average 102µm). However, these values are purely estimates as the depth assessed by confocal may vary with applied pressure on the scope and the depths measured on histology are from one

representative vertical section of the pathology. These results broadly corroborate our ability to accurately assess deep tumour margins and may help refine laser parameters to improve efficacy of ablation.

There were two cases of residual BCC within the ablated area: one with an early nodular component and one that had an infiltrative component. It should be noted that in these two cases, the residual BCC was seen on RCM and it was intentionally decided to proceed with definite excision instead of further ablation, therefore these were not inherent treatment failures. This was performed to verify that our findings on RCM suspicious for residual tumour were in fact BCC on histology and not tissue artifact from ablation. In these cases, we could see the presence of bright tumour islands (enhanced due to aluminum chloride use), elongated nuclei, and clefting. It is important to note that ablated epidermal cells under RCM can sometimes appear elongated, akin to electrocautery effect on histology. This underscores the need for precise ablation and non-excessive fluences. It is ideal to balance the proper amount of thermal destruction to effectively treat tumour while maintaining enough tissue architecture to be able to accurately image with RCM as was demonstrated in this study. Further studies are needed to determine the long-term efficacy of this approach, which we are currently undertaking.

The small sample size and lack of randomization are noted limitations to this study. Because the lesions were excised for detailed histological evaluation of residual tumour, long-term follow-up and assessment for recurrence were not possible. Based on this study, a larger study without surgical excision is warranted to evaluate risk of recurrence after laser treatment alone, as well as to assess cosmesis afterwards.

Similar to a surgical mapping technique, CO₂ laser ablation guided by RCM can eloquently map tumour pre-ablation, detect residual tumour in-between laser passes, and confirm complete destruction post-ablation. This could be an advantageous treatment option for patients who are cosmetically concerned, as CO₂ laser ablation can provide excellent aesthetic outcomes^{10,13}. Other advantages of laser ablation include the precise removal of micron thick skin layers, the low risk of adverse events and short time needed for treatment. Furthermore, in patients with numerous BCCs, multiple comorbidities, or poor coagulation, CO₂ laser ablation could be a viable alternative to surgical excision. The ability to assess for residual tumour in real-time in a non-invasive manner affords no additional morbidity to the patient, allowing for repeat ablation if required and facilitates an efficient and reliable treatment method. Our approach utilizing confocal microscopy could potentially allow for diagnosis, accurate demarcation of tumour margins, and real-time image-guided laser ablation for rapid, comprehensive management of BCCs in one visit.

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What's already known about this topic?

- Non-surgical treatment including laser ablation of skin cancer is limited by lack of histological clearance.
- Reflectance confocal microscopy provides non-invasive, in vivo imaging of skin with cellular-level resolution.

What does this study add?

- Confocal microscopy can eloquently map tumour margins pre-ablation as well as detect residual tumour post-ablation.
- Carbon dioxide laser ablation guided by confocal microscopy may represent a suitable alternative treatment of low risk basal cell carcinoma in non-surgical candidates.

Figure 1.

Pre-ablation Imaging and Assessment of Basal Cell Carcinoma. A) Clinical photograph of a superficial BCC of the left upper back greater than 2cm (Table 1, Case 8). B) Vivablock mosaic (8mm by 8mm) captured at the dermoepidermal junction. Red arrows show areas of tumour clefting and blue arrows show palisading nuclei, features of BCC. There was increased vascularity throughout the lesion (green arrow denotes vessel). C) Enlarged view of the area outlined in red (Fig. 1B) identifying features of BCC including tumour clefting and dilated blood vessels.

Figure 2.

Post-ablation Imaging and Histologic Correlation. A) Clinical photograph of the superficial BCC post-ablation with the CO₂ laser with overlying white char. B) Video mosaic along the edge of the ablated area. C) Enlarged view of the video mosaic showing clearance with the ablated region and features of BCC, including tumour nests (red arrows) and increased vascularity (green arrow) just outside the edge of the area of ablation. D) Histologic correlation with RCM results. Green line denotes the region of ablation. Blue box confirms residual BCC, which was correlated with RCM findings.

Figure 3. Ablation, Imaging, and Histologic Correlation of Basal Cell Carcinoma. A) Pre-ablation clinical photograph of the superficial BCC on the left lower leg (Table 1, Case 5). B) Post-ablation video mosaic. C) Enlarged view of the dermal rings with normal architecture. D) Enlarged view of dermal collagen bundles with scattered inflammatory cells within the ablated region. E) Histology correlation of the ablated area (green line) demonstrating clearance of BCC. Note the preserved epidermis outside the area of ablation.

Table 1**Characteristics of Patients and Lesions with Response to Treatment**

Six of eight lesions showed clearance of the tumour within the area of imaging and ablation using RCM and findings were confirmed with definitive surgical histology. BCC: Basal cell carcinoma; RCM: reflectance confocal microscopy.

Case	Gender	Age	Location of BCC	BCC Subtype	Lesion Size	# Passes	Residual Tumour in Area of Ablation Assessed by RCM	Tumour on Mohs Histology and Vertical Sections within Area of Ablation
1	Female	51	Right post-auricular	Superficial, focal intradermal component	1.0 x 0.9cm	3	No	No
2	Female	68	Left frontal scalp	Superficial and early nodular	1.5 x 1.8cm	2	Yes	Yes
3	Male	35	Left shoulder	Nodular and infiltrative	2.0 x 1.6cm	3	Yes	Yes
4	Male	83	Left upper leg	Superficial	1.0 x 1.0cm	2	No	No
5	Male	83	Left lower leg	Superficial	1.0 x 1.0cm	2	No	No
6	Female	44	Right upper leg	Superficial	1.0 x 1.0cm	3	No	No
7	Female	29	Left upper back	Superficial and nodular	0.5 x 0.5cm	2	No	No
8	Female	93	Left upper back	Superficial	2.3 x 2.0cm	2	No	No