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Research Article

3D Frozen Section Histology - A Technique for Perioperative Complete Evaluation of Surgical Margins in Non-Melanoma Skin Cancer

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Abstract

Background: Basal Cell Carcinoma (BCC) is a highly prevalent skin malignancy often requiring surgical excision. Tumors are frequently located on facial subunits where tissue sparing is a priority. Traditional treatment with excision and bread loaf histology, without frozen sections, samples the surgical margins sparingly, leaving a risk of false negative resection margins. 3D histology is an array of methods aimed at ensuring examination of the entire surgical margin. We present a modified 3D method for BCC on high-risk facial localizations, allowing complete margin control perioperatively, which is practicable in a standard hospital setting without requiring extensive training of personnel.

Methods: The tumor borders are defined and marked. A double-bladed scalpel is used for excision of a circular surgical margin around the entire tumor, and a single blade is used for excision of the tumor. Both are dyed and cryostat sectioned in the pathology lab and examined by the histopathologist.

Cases: Three cases are presented, illustrating the method.

Conclusions: 3D histology techniques have shown high rates of sensitivity, reducing the occurrence of false negative margins and ultimately recurrence rates of BCC, thus reducing the morbidity for patients. Most carcinomas are completely excised in one surgical intervention with this method. The method uses vertical incisions, creating a surgical bed optimized for reconstructive surgery. This 3D histology technique ensures microscopical examination of the entire surgical margin, ensuring the smallest possible defect with radically excised tumor, and is feasible in a standard hospital setting.

Keywords: 3D histology; Basal cell carcinoma; Frozen sections; Microscopically controlled surgery

Introduction

Basal Cell Carcinoma (BCC) is a highly prevalent skin malignancy, with rising incidence worldwide. Surgical excision is the treatment of choice [1]. As tumor extent cannot be determined macroscopically, wide margins are recommended for high-risk BCC (e.g., infiltrative/morphea and micronodular types, recurrent tumor, poorly defined, i.e. difficult-to-treat BCC [2]), while low-risk BCC (superficial and nodular subtypes, well defined) require narrower margins [1]. Most carcinomas occur on sun-exposed areas on the head and neck. As large resections leave large defects, there is a tradeoff between ensuring complete tumor removal and sparing healthy tissue. This is especially true for high-risk and/or poorly defined tumors on facial subunits. In latter cases, intraoperative frozen section examination of surgical margins is often indicated [3]. Surgical incisions can be vertical, horizontal or oblique [4], and histological evaluation of margins can be done with a variety of techniques where the resection margins can be shown in full or in parts. Bread loaf histology with paraffin-embedded tissue is the most widely used histological examination method. However, this method delays microscopy until the following days and only examines a fraction of the margin, leaving a risk of false negative results [4,5].

In poorly defined or high-risk BCCs localized on facial subunits, or when local flaps are required for reconstruction, sample biopsies from the surgical margin and tumor bed can be sent for immediate frozen section examination, increasing the probability of complete surgical excision [6]. In contrast, methods allowing for complete evaluation of surgical margins have been described under the term Microscopically Controlled Surgery (MCS) [7]. These include 1) the Munich method, 2) Mohs micrographic surgery and 3) 3D histology techniques. The Munich method utilizes a cylindrical excision and cryostat technique, followed by multiple horizontal sections allowing examination of the entire surgical margin [8]. In Mohs micrographic surgery (MMS), the tumor is excised obliquely at a 45-degree angle, pressed onto a freeze plate to compress the margin into one horizontal plane, examined, and residual tumor subsequently removed [9]. The excision and histological evaluation are most often performed by a dermatosurgeon trained in Mohs technique, thus performing both the surgery and the histopathologic examination. In an array of techniques, collectively termed 3D histology techniques, vertical sections peripheral to the tumor and horizontal under the tumor are examined, thus providing a complete evaluation of surgical margins [7]. These techniques yield smaller defects allowing for simpler reconstructions, thus sparing healthy tissue, and have shown low recurrence rates [10-12]. First described by Breuninger in 1984 [13], the margin strip method, also termed "Tübinger torte" [12], consists of a vertical incision perpendicular to the skin surface and en bloc removal of the tumor, followed by removal of a thin disc from the entire deep surface of the tumor and a 2 mm strip trimmed from the sides. In the muffin technique [14], after en bloc excision, the tumor is removed from the base and margins, which are then flattened into one plane for histologic evaluation. In other techniques, a marginal strip is first excised for histological examination, and then the tumor is excised; this can be done as the moat or perimeter technique [15], Cockade technique [16], or as the square procedure [17]. In this paper, we describe our modification of the 3D histology technique, in which complete perioperative margin control is ensured using a standard hospital setup. The method is easy to use, provides smaller defects and vertical margins ready for reconstruction, and does not require additional training. We present cases illustrating the method.

Materials and Methods

Patient Selection

Patients of all ages with high-risk BCC localized on facial areas where sparing of a few mm of healthy tissue was a priority were eligible for 3D histology. Patients were informed that we used a variation of the standard frozen section method including examination of the entire margin and base.

Surgical Procedure

In the operating theatre, the surgical field was prepped and draped, and the macroscopic extent of the tumor identified under good light. A surgical margin of 1-2 mm was drawn. Local anesthesia was applied by regional block and/or infiltration. A double-bladed scalpel was used for circular incision around the tumor, placing one blade on each side of the surgical margin. The tumor was excised to the planned depth with a single blade along the inner incision and marked with a suture, usually at the point closest to the vertex, defined as 12 o'clock. The margin strip was excised afterwards to the same depth as the tumor, marked with a suture at 12'o'clock, placed on a cork plate with pins on the inner side of ring specimen, not perforating the tissue (Figure 1). Both tumor and margin were sent to the pathology lab for cryostat processing.

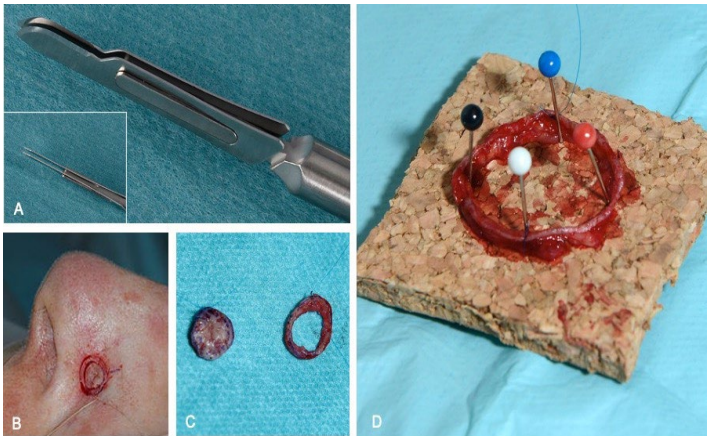

Figure 1: A: The double-bladed scalpel, with 1 or 1.5 mm between the blades; **B**: A circular incision is made with the double-bladed scalpel perpendicular to the skin surface; **C:** The tumor and circular margin strip are marked with a suture and excised using a single-bladed scalpel; **D:** The margin strip is placed on a cork plate with pins on the inner side of ring specimen, not perforating the tissue, thus ensuring that the pathologist can determine the orientation of the margin strip relative to the tumor.

Histopathological Procedure

Tumor: The specimen was examined and described macroscopically, then marked with dye on the deep surface and sides with red color at 12-3 o'clock, blue at 3-6, black at 6-9, and green at 9-12 (Figure 2a). The tumor was then cryo-sectioned from the deep toward the superficial surface, cutting a total of four sections; the first as soon as tissue was seen in the block, the last once when the entire area of the specimen was seen (Figure 2b). The slides were then H&E stained and examined under the microscope.

Margin ring: The circular margin ring was marked with dye in the same way as the tumor (Figure 2c). The ring was divided into 2-4 pieces, depending on the length of the margin strip, and placed in separate cassettes. In the cassette, the margin strip was placed so that the epidermis and dermis were visualized (Figure 2d). The specimens were cryo-sectioned from the outer perimeter inwards in 8 sections, 8m thick at 100m intervals. The slides were H&E stained and examined and the results telephoned to the surgeon, who would re-resect non-clear margins until they were free, then perform reconstruction.

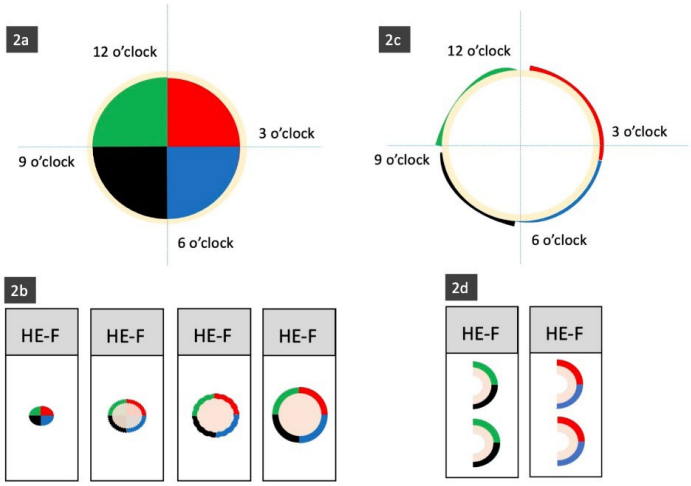

Figure 2: The tumor was marked with dye on the deep surface and sides with red color at 12-3 o'clock, blue at 3-6, black at 6-9, and green at 9-12 **(2a)**. The tumor was cryo-sectioned from the deep toward the superficial surface, cutting a total of four sections; the first as soon as tissue was seen in the block, the last when the entire area of the specimen was seen (i.e., when all colors were represented in the slide) and two in between **(2b)**. The circular margin ring was marked with dye in the same way as the tumor **(2c)**. The ring was divided into 2-4 pieces, depending on size, and placed in separate cassettes. The specimens were cryo-sectioned from the outer perimeter inwards as 8m thick sections at 100m distance, cutting a total of 8 sections for each specimen **(2d)**.

Case Reports

A selection of patients undergoing this modified 3D procedure at our institution is presented below. All patients gave written informed consent to the use of photographs in this publication. Long-term outcomes, including recurrence, will be reported for the whole cohort in a separate publication.

Case 1

A 73-year-old woman presented with recurrent BCC (unknown subtype) on her left ala nasi. Six years previously, the cancer was initially treated by curettage. The following year, the carcinoma recurred, and the patient chose irradiation, receiving 17 fractions of 3 Gy. One year post-irradiation the tumor recurred. The patient was referred for surgery, but instead sought treatment at a private laser clinic, receiving laser treatments every two to four weeks the following three years, before she finally agreed to undergo surgery. Due to prior treatments, a poorly defined area of 3x3 cm with irradiation sequelae and scarring was seen on the left ala. Mapping biopsies were performed, showing nodular BCC in one of nine.

Excision and forehead flap reconstruction under general anesthesia was planned. The 3D technique was used for excision of the most suspicious 3x4 mm of the lesion. Carcinoma was present in the 3-6 o'clock margin, and a re-excision of the 2-7 o'clock margin performed, again as a 1 mm margin taken with the double-bladed scalpel. 3D histology revealed no further carcinoma. As the defect was considerably smaller than anticipated, a simpler bilobed flap was enough for reconstruction (Figure 3).

Figure 3: Case 1 - a 73-year-old woman with recurrent BCC on her left ala, presenting with a 3x3cm area with scarring after multiple laser treatments and curettage. With the 3D technique, a forehead flap was avoided and a simple bilobed flap was used for reconstruction.

Case 2

A 66-year-old male presented with two poorly defined carcinomas on the nose: an 8x8 mm mixed superficial and nodular BCC on the left side of the apex, and a 4x4 mm nodular BCC on the left ala. With traditional treatment, both carcinomas would be excised with a 3 mm surgical margin, leaving a narrow skin bridge between the defects.

The carcinomas were excised with a 1 mm margin and 3D histology technique, using a 1 mm double-bladed scalpel. For the alar BCC, a narrow deep margin was found, and re-excision of the deep margin was performed. All other margins were free of carcinoma, and two rotation flaps were used for reconstruction (Figure 4).

Figure 4: Case 2 - a 66-year-old male with two poorly-defined BCCs on his left ala, excised with the 3D technique and reconstructed with two local flaps.

Case 3

A 54-year-old woman presented with a 7x5 mm BCC of unknown subtype on the left ala nasi, which had been treated with curettage. Using the 3D histology technique, the resultant defect was 9x7 mm. No carcinoma was seen in the surgical margin ring or in the base. The defect was reconstructed with a composite graft from the crus anterior of the left ear helix (Figure 5).

Figure 5: Case 3 - a 54-year-old woman with a poorly defined on her left ala, excised with the 3D technique and reconstructed with a composite graft from the left ear.

Discussion

The aging population and rising incidence of skin cancer demand increasing resources for skin cancer care. It is therefore important to keep costs under control, by using standard methods with simple excision (and reconstruction) for the majority of patients, and more advanced methods such as 3D histology for the subset of patients with high-risk tumors localized in facial subunits where skin sparing is crucial. Applying 3D techniques for a low-risk tumor, e.g., a well-defined nodular BCC localized where tissue sparing is of no concern, is in our view neither feasible nor reasonable. The 3D histology techniques have a range of advantages, the most important being complete evaluation of surgical margins thus minimizing the risk of false negative resection margins. 3D microscopy has been shown to have twice the sensitivity of finding residual tumor compared to standard histological evaluation using the bread loaf technique. Boehringer et al. [18] compared bread loaf sectioning with 3D microscopy in 553 BCCs in a prospective randomized blinded trial. Tumor positive margins were identified in 62/283 (22%) in the bread loaf group, compared to 115/270 (43%) in the 3D microscopy group, which shows that 3D techniques are better at detecting the true involvement of margins.

Lower occurrence of false negative margins also results in reduced recurrence rates. Using 3D histology and formalin-fixed paraffin-embedded tissue processing, Häfner, et al. [10] reported a 5-year recurrence rate of 1% in 3320 BCCs, while Eberle, et al. [11] found a 1.1% recurrence rate in 947 BCCs. Using the cryostat and Tübingen technique, Cecchi, et al. [12] investigated 298 patients with BCC in the head and neck area, reporting an overall recurrence rate of 3% (9/298); 2.2% for primary BCCs and 4.1% for recurrent BCC after a mean follow-up of 5.5 years. In comparison, overall recurrence rates of 3.3% after Mohs micrographic surgery have been reported; 2.1% for primary and 5.2% for recurrent BCC [19]. The Munich method [8] showed 5-year recurrence rates of 3.3% and 7.3% in primary and recurrent BCC, respectively. Recurrence rates after bread loaf histology vary greatly, but have been reported down to 5.9% after primary complete excision [20]. The vertical incision used in 3D microscopy techniques provides an optimal surgical bed for subsequent reconstruction. The 45-degree angle used in Mohs surgery may yield defects with substantial residual subcutis, which often subsequently has to be excised in order to perform reconstruction; a procedure that seems contra-intuitive for a reconstructive surgeon. Multiple resections are often required for total carcinoma clearance in Mohs surgery, with an average of 2.3 stages required in one study of 587 tumors [19]. This increases the duration and cost of the procedure. In contrast, 71% [21], 66% [11] and 55% [10] of BCCs were completely excised in the first stage using 3D techniques.

As the entire margin is examined, a narrow surgical margin can be used, thus sparing healthy tissue. This results in smaller defects and allows for simpler reconstructive methods as illustrated in the first case presented, where a complex multi-stage forehead flap reconstruction was avoided due to the use of 3D histology, and a smaller and simpler 1-stage local flap reconstruction was sufficient (Figure 3). In addition, the 3D microscopy method allows the highest level of specialist expertise to perform each

their specialized subsets of tasks: the reconstructive surgeon performs the excision and reconstruction and the pathologist the histopathological evaluation. This permits the method to be implemented in any hospital setting, without requiring additional training of personnel. A single-bladed scalpel may be used, but we found that the resection margin strip was more even when the double-blade was used, and this permits precise standardized surgical excision of a narrow strip. The double-bladed scalpel can be provided with different distances between the blades. A double-bladed scalpel with an inter-blade distance of 1 mm was initially used for excisions in all locations, including periorbital, nose and perioral areas. However, in thin and frail skin, the 1mm margin strip is difficult to prepare for histopathological evaluation. We then switched to a 1.5 mm double-bladed scalpel, finding the quality of the margin strip better for histological preparation. However, we found that eyelid skin is too thin for this technique.

Disadvantages of 3D histology compared to the traditional bread loaf technique are longer time for tissue preparation and histopathological examination. There are some extra costs in the laboratory as the procedure is more time-consuming. Currently, we find the technique indicated in high-risk carcinomas located in areas where tissue sparing is of high priority and less than 2 cm in diameter.

Conclusion

3D histology ensures microscopical examination of the entire surgical margin, producing the smallest possible defect with radically excised tumor, leaves defects ready for reconstruction, and is feasible in a standard hospital setting without requiring extensive training of personnel.

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