Effect of 27-MHz Radiofrequency on Hair Follicles: Histological Evaluation of Skin Treated Ex Vivo

Dong Hyun Kim, MD,*†‡ Amélie Lavoie, MSc,*† Gilles Ratté, MSc,§ Clément Beaumont,§ Lucie Germain, PhD,*† and Danielle Larouche, PhD*†

BACKGROUND A multitude of methods and treatments exist for cosmetic hair removal. Electroepilation is a commonly performed method of hair removal that is so-called "permanent"; however, there is a paucity of histological studies of the effects of radiofrequency (RF) on hair follicles.

OBJECTIVE This study aimed to observe the destruction of human hair follicles and surrounding tissue after the treatment with 27.12-MHz RF, with more attention paid to the thermal destruction of bulge and bulb/dermal papilla.

METHODS Human scalp specimens obtained during face-lift surgery were treated with 27.12-MHz RF. The probe tip was inserted into hair follicle, RF current was applied, and treated specimens were processed for histological analysis.

RESULTS Significant damages were observed on treated hair follicles. Thermal damage was lance-shaped and extended over several hundred micrometers (100–400 μm). The location of destruction areas varied, likely depending on the point of insertion of the probe. The epidermis remained intact.

CONCLUSION This study shows that the general mechanism of thermolysis is to generate damage to cells and tissues surrounding the insertion point of the filament. The results suggest that if the insertion point is close to the bulge region, there is a risk to destroy hair follicle epithelial stem cells.

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The presence of unwanted hair on face and body is a common problem that causes a particular interest for the development of advanced treatment. A wide range of methods about cosmetic hair removal or reduction is available. Among them, there are 2 methods working through follicular destruction or inhibition of growth cycle; electroepilation and light-based hair removal (LHR).[1]

Since 1996 when Grossman and colleagues[2] first reported the photothermal destruction of pigmented hair follicles by a ruby laser, numerous advances have been made in LHR. On the basis of the theory of selective photothermolysis,[3] the efficacy and safety of LHR depend largely on hair type and skin color. People with dark hair and light skin are ideal candidates for LHR, whereas darker skin has a tendency to get epidermal damage leading to burns and dyspigmentation. To date, most of LHR devices produce a partial reduction in hair growth and temporary hair loss. Hair regrowth on their treated areas is frequently encountered, which indicates the interruption of the

Centre de recherche en organogénèse expérimentale de l'Université Laval/LOEX, Centre de recherche du Centre Hospitalier Universitaire (CHU) de Québec, Axe médecine régénératrice, Québec, Canada;†Département de Chirurgie, Faculté de Médecine, Université Laval, Québec, Canada;‡Department of Dermatology, CHA Bundang Medical Center, CHA University, Seongnam, Korea;§Dectro International, Québec, Canada

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hair cycle and the re-emergence of anagen hair.[4] In this case, it may be useful to remove remaining hairs by electroepilation.

Electroepilation includes electrolysis (direct current), thermolysis (radiofrequency [RF]), and a combination of both. Different from optic energy, electroepilation is dependent on the electrical properties of the tissue rather than on concentration of chromophores in the skin for thermal destruction of hair follicles.[5] The method of electrolysis involves the insertion of a small needle or probe into the hair follicle through which an electric current is delivered. In thermolysis, RF energy emanates from the probe tip to tissue and heats the hair follicles over 60°C (140°F) but is barely perceptible at the skin surface.[6] The range of designated frequencies used for thermolysis has been increasing since 1925 from 0.5, 1.7, 3.35, and 4 MHz, then in 1985, the Federal Communications Commission introduced rules for industrial, scientific, and medical ISM applications with frequencies of 6.78 MHz and 13.56 to 27.12 MHz. Thermal sensation perceived by the patient is generally less painful with increasing frequency. Although electroepilation is a commonly performed procedure in practice, there is a paucity of histological studies on the effects of RF on hair follicles, in particular effects of the 27.12-MHz RF.

This study aimed to observe the histomorphologic change of human hair follicle and surrounding tissue after the treatment of 27.12-MHz RF on ex vivo human scalp specimen. Optical microscopic observations reported here allow to better understand the effect of RF on hair follicles and to add scientific evidence providing possible explanations for the clinical response in practice.

Materials and Methods

Human Skin Sampling

The study was approved by the "comité d'éthique de la recherche du Centre hospitalier universitaire (CHU) de Québec" for the protection of human subjects. All skin samples were obtained after informed consent was given. Experiments were conducted on adult scalp specimens from patients with Fitzpatrick skin Type II removed during face-lift surgery. Each experiment with different parameters was tested on the skin of at least 2 different donors. After harvesting, skin samples were conserved overnight at 4°C in a solution of Dulbecco's modified Eagle's medium with Ham's F12 medium in a 3:1 proportion, supplemented with 24.3 mg/L adenine, 100 IU/mL penicillin G, 25 μg/mL gentamicin, and 0.5 μg/mL amphotericin B. After phosphate-buffered saline washings and tempering at room temperature, hair-bearing parts were cut into approximately 1 cm wide by 2.0 cm long strips. Specimen strips were deposited on a conductive plate so that RF current can flow through the specimen. All samples were treated within 24 hours after harvesting.

Thermolysis

Thermolysis was performed using Apilus platinum device (Dectro International, Québec, Canada), which emanates 27.12-MHz RF. The half-insulated flexible probe was inserted into hair follicle until the probe tip touched the bottom of hair follicle and retreated a little bit to attempt to relocate the probe tip between the bulge and the bulb. Single RF current was applied into many hair follicles as possible, and treated specimens were processed for further histological analysis. For each donor, an untreated skin sample was set aside for biopsy. According to the Apilus platinum pratical guidelines, various parameters were used in the experiment, including the operation mode (PicoFlash mode: pulses in thousandth of a second and Multiplex mode: slow heating followed by PicoFlash mode), the probe (F1ITH: 3 mm, F3ITH: 5 mm, and F5ITH: 7 mm in length), total duration of impulses in Multiplex mode, percent intensity of heating, number of insertion, duration of each impulse, and percent intensity of each impulses. Peak temperature in the skin at the moment of RF treatment approximates 75°C (167°F) (manufacturer's data). Each skin strip was cut into 4 to 6 smaller pieces and fixed with Histochoice MB fixative (Amresco, Solon, OH) and embedded in paraffin for further histological analyses.

Histological Analysis

To analyze the destruction of hair follicles after thermolysis, biopsies were fixed in Histochoice MB

(Amresco) and embedded in paraffin. Sections of 10 μm were stained with hematoxylin–phloxine–saffron or Masson trichrome using Weigert hematoxylin, fuchsin-ponceau, and aniline blue stains. The slides were examined under an AxioImager M2 equipped with a AxioCam ICc1 (Zeiss, Toronto, ON, Canada) digital camera for color pictures.

Results

A total of 48 sessions of treatments with 27.12-MHz RF were performed ex vivo on scalp specimens using various parameters and intensities (Table 1). Fourteen sections showing entire hair follicles were selected for the histological analyses of the destruction of hair follicles and surrounding tissue (Table 1).

Compared with control hair follicle (Figure 1A), most treated hair follicles showed clear and definite damaged area, with missing or torn apart hair shaft in hematoxylin–phloxine–saffron stain (Figure 1B–E). Often, follicular damage was observed in the superior third (infundibulum) and middle (isthmus) of the hair follicles (Figure 1C–E). On the hair follicle presented on Figure 1C, damages extend over a radius of several hundred microns (up to 250 μm) (Figure 1C, brace) and include important epithelial cell alterations and damage to surrounding dermal tissue (Figure 1C, black arrow). However, hair bulb and dermal papilla were not damaged (Figure 1C, white arrow) despite large destruction of upper part. Sometimes, follicular alterations were observed in one side of the hair follicle (Figure 1E, white asterisk) and not in the other side. Destruction signs were also observed in the bulb/dermal papilla (Figure 1B, arrowhead). In all tested conditions, the epidermis appeared intact.

TABLE 1. Rating of Observed Effects for Each Tested Parameter

								Rating				
No	*Mode*	*Type of Filament*	*T Total (sec)*	*H (%)*	*N*	*T Imp (sec)*	*Int. Imp (%)*	*1/3 sup*	*Bulge*	*Papilla*	*Thermal Damage Radius (μm)*	*Figures*
1			No treatment						Normal			Figure 1A
2	P	F1ITH	0.0	0	1	0.08	70	++	–	–		
3	P	F3ITH	0.0	0	1	0.08	55	+++	–	–		Figure 1E
4	P	F3ITH	0.0	0	1	0.08	55	+++	+++	–		
5	P	F3ITH	0.0	0	2	0.08	70	+++	+++	–	307.9	Figure 1B,C
6	P	F3ITH	0.0	0	1	0.10	70	+++	+++	–		Figure 1D
7	P	F3ITH	0.0	0	1	0.08	70	++	++	–		
8	P	F5ITH	0.0	0	1	0.08	70	+++	–	–		
9	M	F3ITH	0.5	10	1	0.08	70	++	++	–	116.3	Figure 3A
10	M	F3ITH	1.0	10	1	0.08	70	+++	–	–	359.3	
11	M	F3ITH	1.0	15	1	0.08	70	++	++	–	249.1	Figure 2
12	M	F3ITH	1.0	15	1	0.08	70	++	++	–		
13	M	F3ITH	1.0	15	1	0.08	70	++	++	+	387.7	Figure 3B
14	M	F3ITH	2.0	10	1	0.08	70	++	++	+	246.2	
											277.8 ± 97.5	

H, percent of intensity of heating; Int imp, percent of intensity of each impulse; M, MultiPlex mode; N, number of insertion; P, PicoFlash mode; rating, rating of observed effects (– = no effects, + = low, ++ = moderate, +++ = high); T total, total duration of impulses in MultiPlex mode; T imp, duration of each impulse; 1/3 sup, the superior third of the hair follicle (F1ITH: 3 mm, F3ITH: 5 mm, and F5ITH: 7 mm in length).

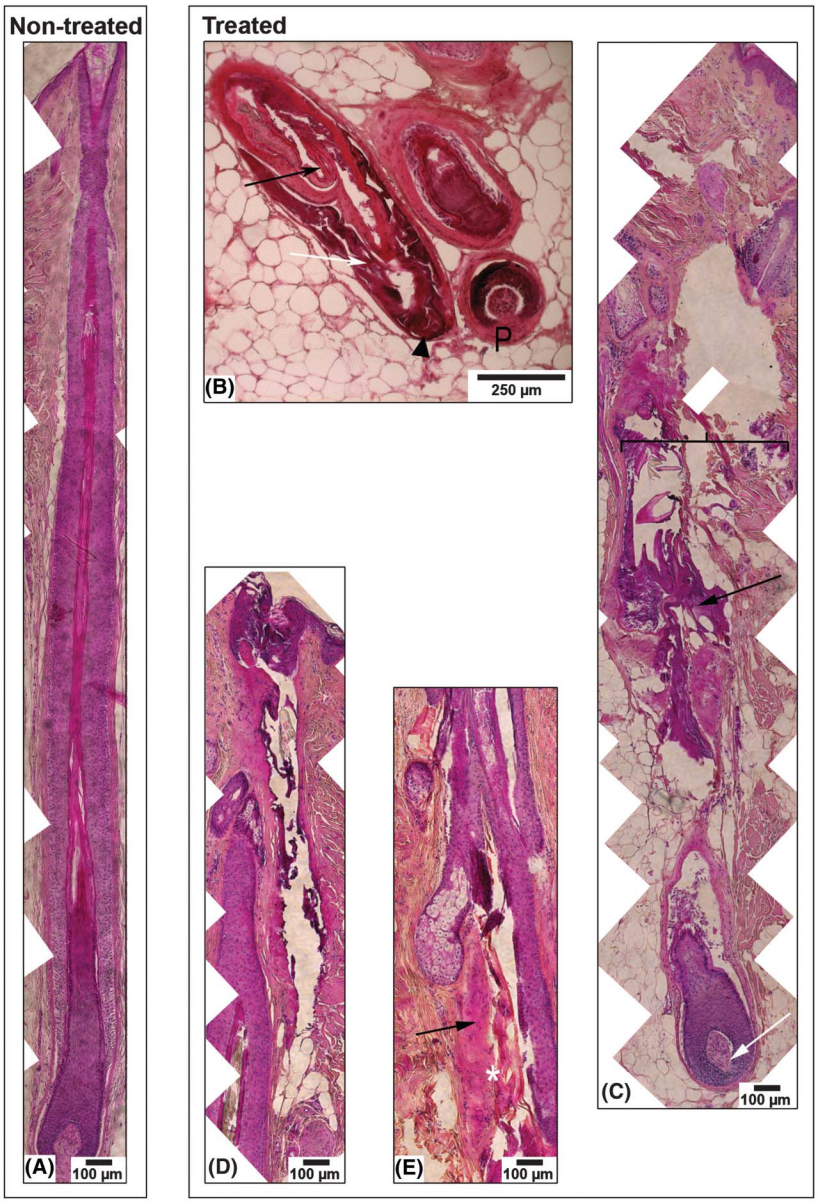

Figure 1. Histological evaluation of follicular destruction after 27-MHz RF treatment using the Apilus platinum device (hematoxylin–phloxine–saffron stain). Hair follicle of a 62-year-old woman (A–C) and 66-year-old woman (E) untreated (A) or treated (B–E) with a F3ITH electrolysis filament using the PicoFlash mode. Refer to Table 1 for additional details about settings used. Note important cellular damages to the hair shaft (black arrow) and epithelial cells (white arrow) in a treated hair follicle (B) and that the dermal papilla, which appears normal on the right follicle (P), seems completely destroyed on the left follicle (arrowhead). Note thermal damage located mainly in the superior third of the hair follicles seen in (C–E). Note damages extending over a radius of several hundred microns (up to 250 µm on picture C [brace]). Note destruction of all follicular epithelial cells on one side of the left follicle of E (arrow). Note destruction of hair shaft and inner root sheath in one side of hair follicle on E (asterisk). Scale bars: 100 µm. P, dermal papilla.

To better evaluate damage to dermal tissue, Masson trichrome stain was performed because heat-denatured collagen stains red instead of blue with this stain.[7,8] As expected, red spots (Figure 2, white arrowheads) were observed in dermal connective tissue surrounding hair follicles presenting cellular breaks (Figure 2, white arrow), indicating the extent of heat diffusion. Collateral thermal damage was sometimes observed on sebaceous glands (Figure 2, red arrows) where sebaceous glands appeared to be shrinking and necrotic. Overall, thermal damage zone was lance-shaped (Figure 3, dotted area) and extended over a mean

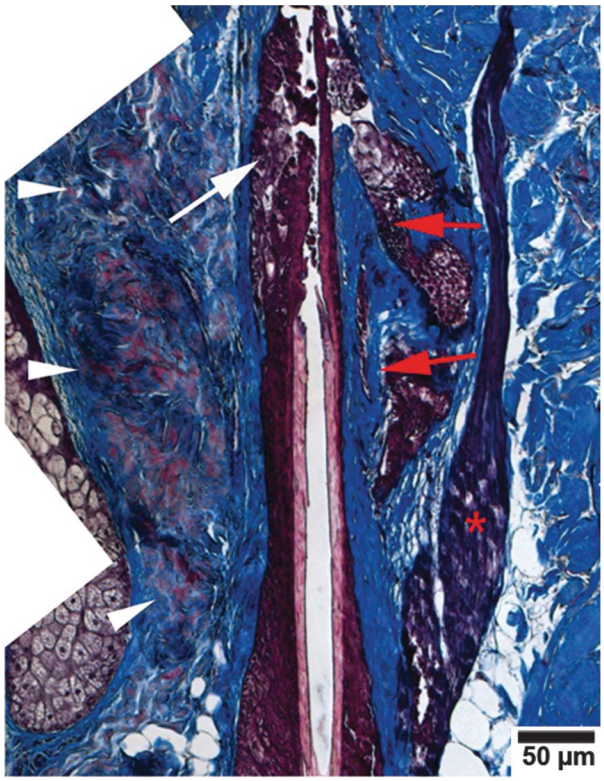

Figure 2. Evaluation of collateral thermal damage in Masson trichrome stain. Note that some of the conjunctive tissue appeared red rather than blue in Masson trichrome stain (white arrowheads), suggesting heat denaturation. Note destruction signs on hair follicle epithelial cells (white arrow), sebaceous gland (red arrows) and arrector pili muscle (red asterisk).

radius of 277.8 ± 97.5 µm from the center of the insertion tip (Table 1).

Discussion

In this study, 27.12-MHz RF was applied to human scalp specimens with various parameters, and histological analyses were performed. Resulting observations allowed us to understand the immediate histological effects of thermolysis using 27.12-MHz RF.

Thermolysis is considered as a permanent hair removal method, which irreversibly destruct hair follicles.[9] Through interaction with epithelial stem cells, which reside in the bulge area,[10] dermal papilla cells orchestrate cyclic regeneration of hair follicles and are responsible for the constant regeneration of hairs.[11,12] Thus, if dermal papilla is efficiently destructed, it may not be necessary to destruct the bulge region that houses a reservoir of epithelial stem cells that are also paramount for the renewal of the epidermis and its repair after wounding.[13,14] In this study, the authors observed that zones presenting heat-diffusion signs were likely limited to the proximity of the probe and mainly located in the upper and mid portion of hair follicles. Dermal papillae often remained intact after treatment. During the procedure, the probe was inserted into hair follicle until the probe tip touched the bottom of hair follicle and retracted a little bit to attempt to relocate the probe tip between the bulge and the bulb. Given the considerable length of scalp hair follicles and the unusual tissue tension within skin samples, the probe was probably retracted to far from the bulb to destroy dermal papilla in many cases. Therefore, it is probable why the damages were narrowed to upper part in some hair follicles under our in vitro experimental conditions. However, the size distribution of the heat-diffusion zone observed allow us to extrapolate that a correctly inserted electrolysis filament to the bottom of hair follicle leads to dermal papilla destruction.

In this study, the authors adopted histomorphology to verify the integrity of the tissue architecture after ex vivo thermolysis treatment on skin specimens. The principal limitation associated to ex vivo study is that cell viability does not mimic the in vivo situation. However, it was well documented that skin harvested from living persons remains viable for several hours. In the untreated group with same time lapse between fresh skin tissue sampling and fixation as the thermolysis-treated group, histological analyses showed a good preservation of tissue architecture without any sign of cellular damage, including epidermal vacuolization and degeneration. So, it can be assumed that our study protocol allowed minimal damage of cell viability. However, observed damages in the thermolysis-treated group are not necessarily associated to cell mortality. Alternatively, even if the bulb is destroyed, heat can transfer to the bulge and kill cells, even if this does not show up on routine staining. More precise measurement of cell viability would be required to address this question.

Radiofrequency was known to produce a highly efficient thermal effect on hair follicles.[5,15] A related adverse effect is pain accompanying the procedure

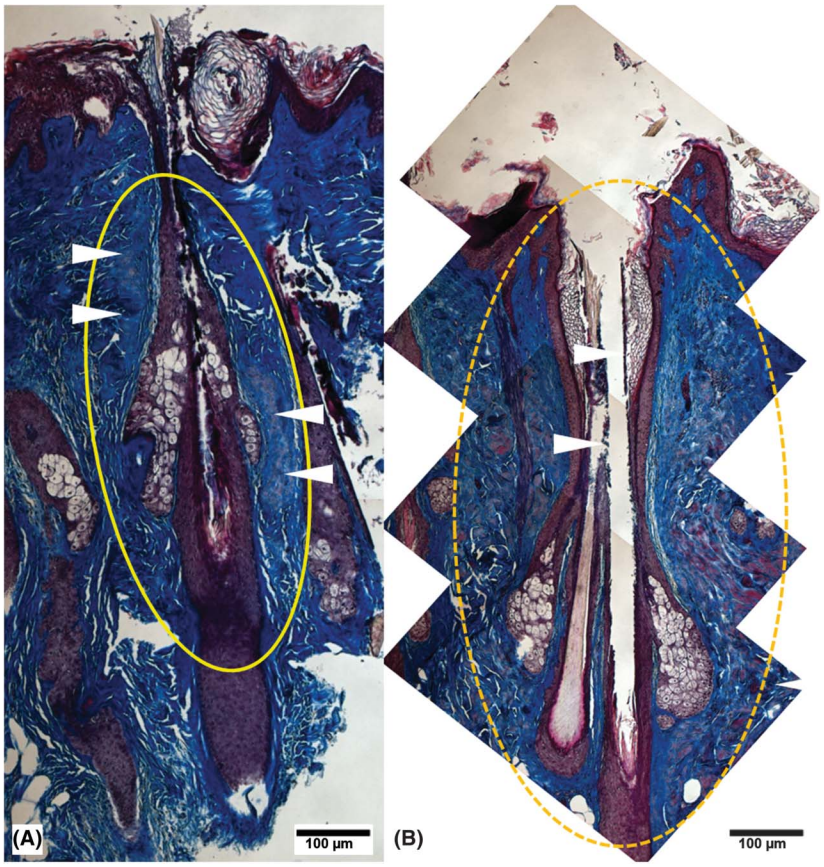

Figure 3. Lance-shaped collateral thermal damage in thermolysis-treated hair follicle (Masson trichrome stain). Overall, thermal damage zone, where reddish connective tissue was observed (white arrowheads), was lance shaped (A, B, area bounded by yellow) and extended over several hundred micrometers in radius from the center of the insertion tip.

and the risk of scar that can happen when the dermis is damaged.[16] New thermolysis devices have precise automatic timers and insulated probes that reduce the risks of scarring. There are devices that generate different RF, the most common is 13.56-MHz. In this study, the authors used a device generating 27.12-MHz RF, which has several advantages over conventional 13.56-MHz RF. Because the sensation perceived by the patient seems to be less intense as the frequency increases, a frequency of 27.12-MHz will be better for pain control. Also, the 27.12-MHz frequency has a more efficient power absorption by water molecules, meaning that less power is needed to epilate a hair. Furthermore, rapid positive to negative polarity changes, 27 million cycles per second, allows more precise and fast electrocoagulation. In this study, Masson trichrome stain revealed that thermal damages after thermolysis treatment are lance shaped, and thermal diffusion might extend over about 300 µm (277.8 ± 97.5 µm, $n = 6$) around the hair follicles, thus reaching the dermis. Because RF energy emanates from the probe tip, this observation confirms that thermolysis is a highly skilled procedure that centers around accurate probe insertion, coupled with the correct dosage of current to permanently remove hair while avoiding the risk of dermal injury.

In summary, clinical observations show that thermolysis is an effective method to permanently destroy hair follicle and has the advantages of acting independently of hairs or skin color. This study shows that the general mechanism of thermolysis is to destroy cells and tissues surrounding the insertion point of the filament and emphasizes the fact that skill of the therapist is of great importance because only a proper insertion of the filament to the bottom of the hair follicle can reach the dermal papilla cells responsible of the hair regrowth.

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Address correspondence and reprint requests to: Danielle Larouche, PhD, CMDGT/LOEX Aile-R, CHU de Québec, 1401, 18e rue, Québec, Québec, Canada, G1J 1Z4, or e-mail: danielle.larouche.2@ulaval.ca