

# Histological in vitro evaluation of the effect of 27-MHz radiofrequency on ex vivo hair follicles

**Running head:** The effect of 27-MHz radiofrequency on hair follicle

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## **Abstract**

**Background** A multitude of methods and treatments exist for cosmetic hair removal. Since the 1990s, laser hair removal stands foremost as a quicker and easier way to reduce unwanted hair. Electro-epilation is a commonly performed method of hair removal that is permitted to be called 'permanent', however there is a paucity of histological studies of the effects of radiofrequency (RF) on hair follicles.

**Objectives** This study aimed to observe the destruction of human hair follicles and surrounding tissue following the treatment with 27-MHz RF, a frequency generating less pain compared to the most common used 13.56 MHz, 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-MHz RF. The probe tip was inserted into hair follicle and RF current was applied. Various combinations of parameters were tested and the specimens with the destruction of hair follicles were chosen for histological analysis.

**Results** Significant damage was observed in the hair follicles. Thermal damage was lance-shaped and extended over several hundred micrometers (100-400  $\mu\text{m}$ ). The location of destruction areas varied, likely depending of the point of insertion of the probe. Epidermis remained intact due to the use of insulated probes.

**Conclusions** The general mechanism of thermolysis is to generate thermal destruction of the hair follicles, which is limited to within close proximity of the insertion point of the probe.

Our results suggest that if the insertion point is close to the bulge region, there is a risk to permanently destroy epithelial stem cells and that the dermal papilla destruction, required to stop hair regrowth, depends on the proper insertion of the probe to the follicle bottom.

## Introduction

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 two methods working through follicular destruction or inhibition of growth cycle; electro-epilation and light based hair removal (LHR).<sup>1</sup>

Since 1996 when Grossman et al.<sup>2</sup> 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<sup>3</sup>, 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, while darker skin has a tendency of epidermal damage leading burns and dyspigmentation. To date, most of LHR devices produce a partial reduction in hair growth and temporary hair loss. Hair re-growth on their treated areas is frequently encountered, which indicates the interruption of the hair cycle and the re-emergence of anagen hair.<sup>4</sup> In this case, it may be useful to remove the remaining hair by electro-epilation.

Electro-epilation includes electrolysis (direct current), thermolysis (radiofrequency, RF), and a combination of both. Different from optic energy, electro-epilation is dependent on the electrical properties of the tissue rather than on concentration of chromophores in the skin for thermal destruction of hair follicles.<sup>5</sup> 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.<sup>6</sup> The range of designated frequencies used for thermolysis has been increasing from 1.7-MHz, 3.35-MHz, 6.75-MHz, 13.56-MHz to 27-MHz. Thermal sensation perceived by the patient is generally less painful with increasing frequency. Although electro-epilation is a commonly performed procedure in practice, there is a paucity of histological studies of the effects of RF on hair follicles.

The present study aimed to observe the destruction of human hair follicle and surrounding tissue following the treatment of 27-MHz RF on *ex vivo* human scalp specimen. The histological observation allows 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 two patients with Fitzpatrick skin type II during face-lift surgery. Each experiment with different parameters was tested on the skin of at least two different donors. After phosphate buffered saline (PBS) washings, hair-bearing parts were shaved and 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.

### **Thermolysis**

Thermolysis was performed using Apilus platinum device (Dectro International, Québec, Canada), which emanates 27-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 re-locate the probe tip between the bulge and the bulb. Single RF current was applied into the hair follicles and each hair follicle was marked with for the further histological analysis. For each donor, an untreated skin sample was set aside for biopsy. According to manufacturer's practical guideline, various parameters were used in the experiment, including the operation mode (PicoFlash<sup>TM</sup> mode; pulses in thousandth in a second, Multiplex<sup>TM</sup> mode; slow heating followed by PicoFlash<sup>TM</sup> mode), the probe (F11TH;

3mm, F3ITH; 5mm, F5ITH; 7mm in length), total duration of impulses in MultiPlex mode, % intensity of heating, number of insertion, duration of each impulse, % 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 in to 4-6 smaller pieces and processed for histological analyses.

### **Histological analysis**

To analyze the destruction of hair follicle after thermolysis, biopsies were fixed in Histochoice™ MB® (Amresco, Solon, Ohio) and embedded in paraffin. Sections of 10 µm were stained with hematoxylin-phloxine-saffron and Masson's trichrome using Weigert's hematoxylin, fuchsin-ponceau and aniline blue stains. Because heat-denatured collagen in burned skin stains red instead of blue in Masson's trichrome stain, collateral thermal damage was measured as a red spot in the surrounding dermal connective tissue.<sup>7,8</sup> The slides were examined under an AxioImager M2 equipped with a AxioCam ICc1 (Zeiss, Toronto, Ontario, Canada) digital camera for color pictures.

## Results

A total of 48 sessions of treatments with 27-MHz RF were performed ex vivo on scalp specimens using various parameters and intensities (for details, see 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 to control hair follicle (Fig. 1a), most treated hair follicles showed clear and definite destruction area, with missing or torn apart hair shaft in hematoxylin-phloxine-saffron stain (Fig. 1b-e). Often, follicular damage was observed in the superior third (infundibulum) and middle (isthmus) of the hair follicles (see Fig 1c,d,e for examples). On the hair follicle presented on Fig. 1c, damages extend over a radius of several hundred microns (up to 250  $\mu\text{m}$ ) (Fig. 1c, brace), and include important epithelial cell destruction and damage to surrounding dermal tissue (Fig. 1c, black arrow). However hair bulb and dermal papilla were not damaged in spite of large destruction of upper part (Fig 1c, white arrow). Sometimes, follicular destruction was observed in one side of the hair follicle (Fig. 1e, white asterisk). Destruction was also observed in the bulb/dermal papilla (Fig 1b, arrowhead) in the case that the probe reached the bottom of hair follicles. In all tested conditions, epidermis remained intact.

To better evaluate damage to dermal tissue, Masson's trichrome stain was performed, because heat-denatured collagen stains red instead of blue.<sup>7,8</sup> As expected, red spots (Fig. 2, white arrowheads) were observed in dermal connective tissue surrounding hair follicles presenting cellular breaks (Fig. 2, white arrow) indicating the extend of heat diffusion.



Collateral thermal damage was sometimes observed on sebaceous glands (Fig. 2, red arrows) where sebaceous glands appeared to be shrinking and necrotic. Overall thermal damage zone was lance-shaped (Fig. 3, dotted area) and extended over several hundred micrometers ( $277.8 \pm 97.5 \mu\text{m}$ , Table 1).

## Discussion

In this study, 27-MHz radiofrequency 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-MHz RF.

Thermolysis is considered as a permanent hair removal method, which irreversibly destruct hair follicles.<sup>9</sup> Through interaction with epithelial stem cells, which reside in the bulge area<sup>10</sup>, dermal papilla cells orchestrate cyclic regeneration of hair follicles and are responsible for the constant regeneration of hairs.<sup>11,12</sup> Thus, if dermal papilla is efficiently destructed, it may be not 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.<sup>13,14</sup> In this study, we observed that zones presenting heat diffusion signs was 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 following treatment. Given the considerable length of scalp hair follicles and the unusual tissue tension within skin samples, it was difficult to insert the probe to the bottom of the follicle as it is usually done in clinical. Therefore, it is probably why the damages were narrowed to upper part in some follicles. 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.

RF was known to produce a highly efficient thermal effect on hair follicles.<sup>5,15</sup> A related adverse effect is pain accompanying the procedure and the risk of scar that can happen when

dermis is damaged.<sup>16</sup> 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, we used a device generating 27-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-MHz will be better for pain control. Also, the 27-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's trichrome stain revealed that thermal damages following thermolysis treatment are lance-shaped and thermal diffusion might extend over a 7.7 mm<sup>2</sup>-lance-shaped area 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 centres around accurate probe insertion, coupled with the correct dosage of current in order 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 emphasize the fact that skill of the therapist is of great importance since 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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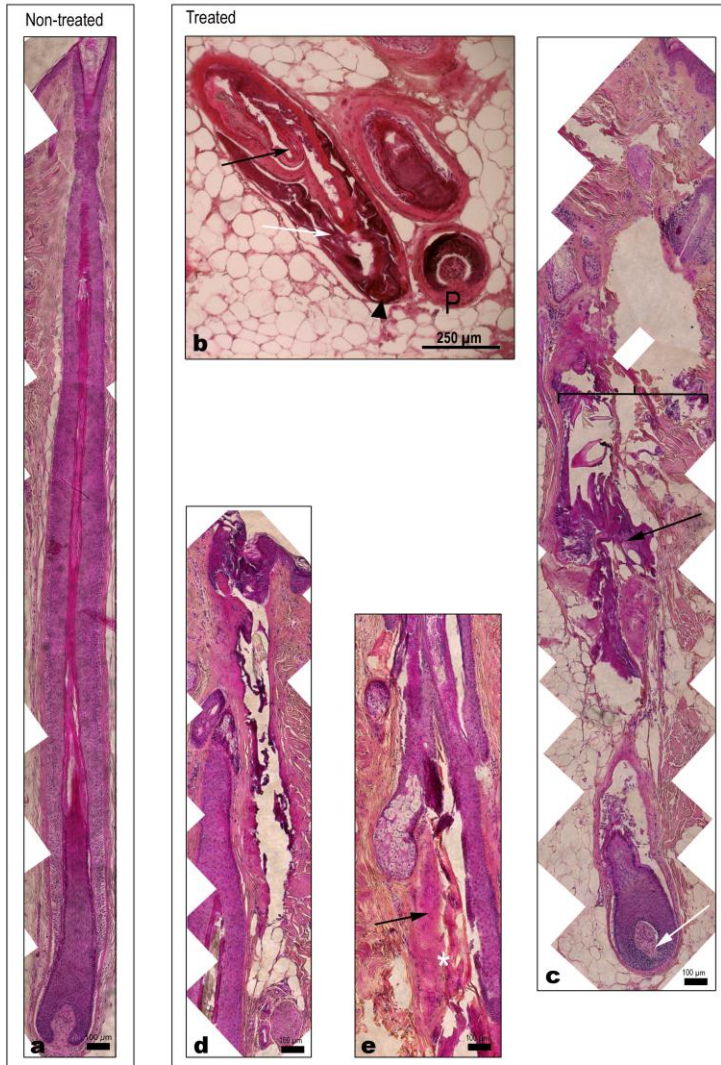
**Table 1. Rating of observed effects for each tested parameter**

No	Mode	Type of filament	T total (sec)	H (%)	N	T imp (sec)	Int. imp (%)	Rating*			Thermal damage (µm)	Figures
								1/3 sup	Bulge	Papilla		
1			No treatment					Normal				Fig. 1a
2	P	F1ITH	0.0	0	1	0.08	70	++	-	-		
3	P	F3ITH	0.0	0	1	0.08	55	+++	-	-		Fig. 1e
4	P	F3ITH	0.0	0	1	0.08	55	+++	+++	-		
5	P	F3ITH	0.0	0	2	0.08	70	+++	+++	-	307.9	Fig. 1b,c
6	P	F3ITH	0.0	0	1	0.10	70	+++	+++	-		Fig. 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	Fig. 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	Fig. 2
12	M	F3ITH	1.0	15	1	0.08	70	++	++	-		Fig. 3b
13	M	F3ITH	1.0	15	1	0.08	70	++	++	+	387.7	
14	M	F3ITH	2.0	10	1	0.08	70	++	++	+	246.2	
											277.8 ± 97.5	

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

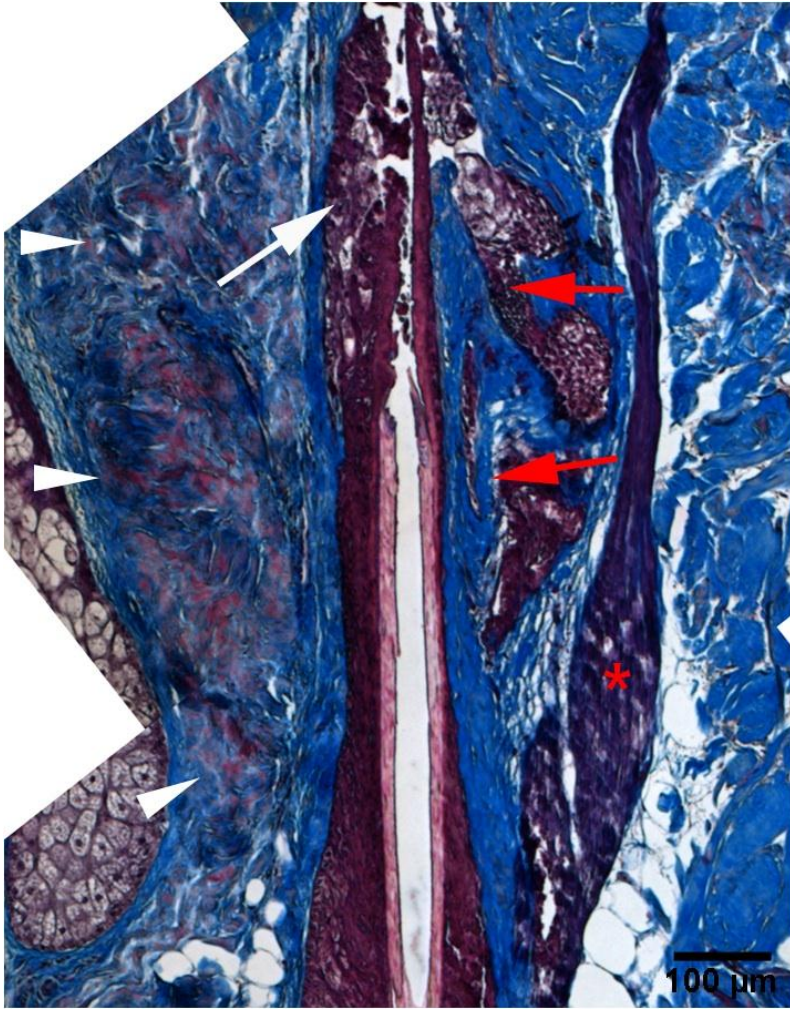


## Figure legends

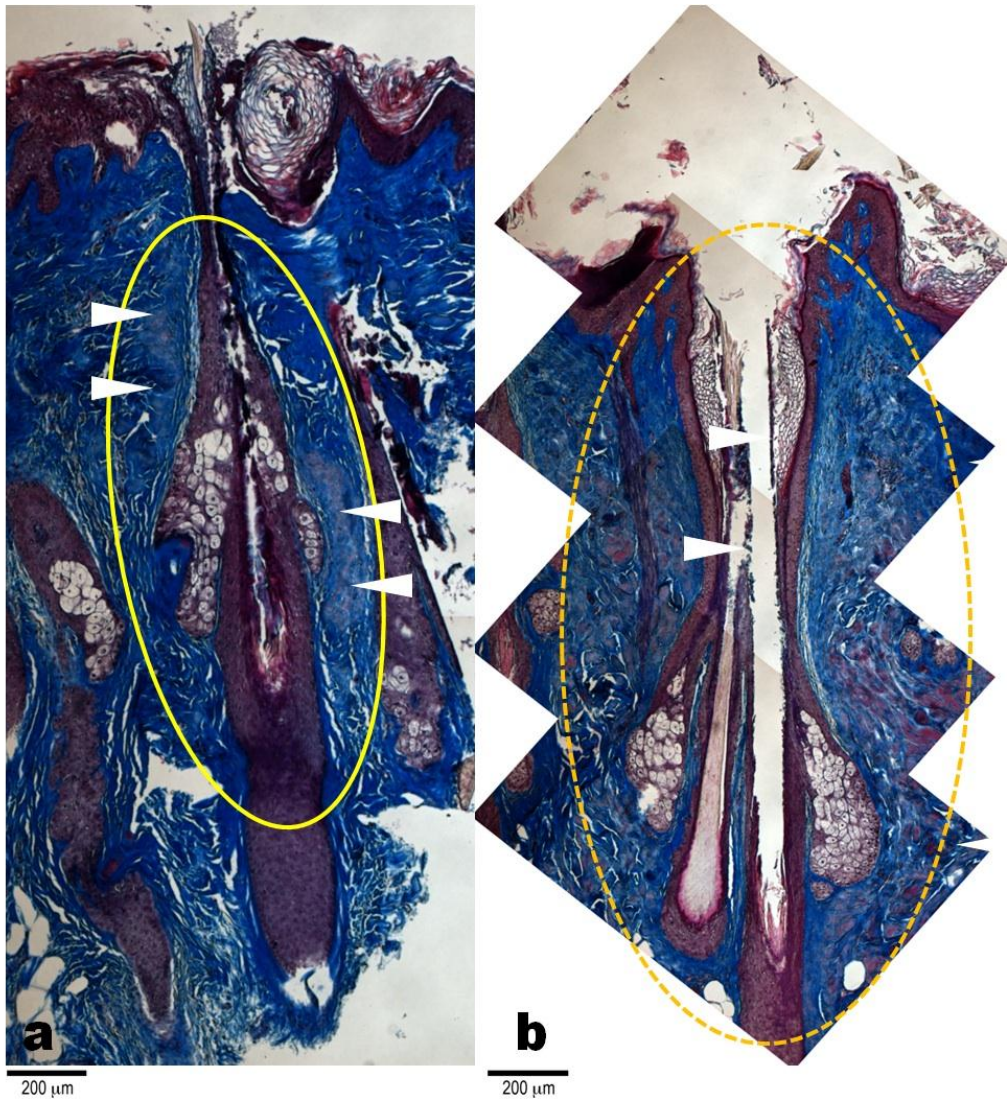


**Fig. 1.** Histologic evaluation of follicular destruction following 27-MHz RF treatment using the Apilus Platinum device. (hematoxylin-phloxin-saffron stain. Hair follicle of a 62 (a-c) and 66-year-old woman (e) untreated (a) or treated (b-e) with a F3ITH electrolysis filament using the PicoFlash mode. 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 right follicle (P), seems completely destroyed on left follicle (arrowhead). Note thermal damage located mainly in the superior third of the hair follicles seen in c, d and e. Note damages extending over a radius of several hundred

microns (up to 250  $\mu\text{m}$  on picture c (braces). Note destruction of all follicular epithelial cells on picture e (arrow). Note destruction of hair shaft and inner root sheath in one side of hair follicle on picture e (asterisk). Scale bars: 100  $\mu\text{m}$ . P: dermal papilla .



**Fig. 2.** Evaluation of collateral thermal damage in Masson's trichrome stain. Heat-denatured collagen stains red instead of blue in Masson's trichrome stain (white arrowhead) (red asterisk; arrector pili muscle).



**Fig. 3.** Lance-shaped collateral thermal damage in thermolysis-treated hair follicle. . Overall thermal damage zone was lance-shaped (dotted area) and extended over several hundred micrometers in radius ( $277.8 \pm 97.5 \mu\text{m}$ ).