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RESEARCH ARTICLE

Histopathologic Comparison of Radiofrequency and Scalpel Blade on Mice Skin Incisional Wound Healing Model

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ABSTRACT

Using a safe and accurate alternative to scalpel blades for various surgical operations has been more considering today. There are many studies about the healing process of various tissues after using different methods like radiofrequency, electro surgery and scalpel blade for the surgical incisions, but choosing the right instrument is very important in cosmetic surgeries.

This study was undertaken to get more histopathologic details about the healing process after using either Radiofrequency (RF) or Scalpel blade as a skin incision method. So, an experimental study was designed in that 12 mice were randomly divided in three groups with the same situation and two different incisions were cut in each mouse on ventral skin by scalpel blade and radio surgical equipment. The skin specimens from scalpel blade incision as group one sample and radiosurgical incision as group two sample were harvested on the same operation day and a weekly manner to day 21. All samples were stained with Hematoxillin - Eosin and observed under light microscopy. Results showed less tissue contraction at the cutting edge and better collagen alignments in RF incision in comparison to scalpel blade incisions in which having fibrosis and more tissue contraction. These histopathologic findings might be the reason for more elasticity and less scar tissue remaining after skin Radiofrequency surgery.

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INTRODUCTION

There has been a lot of interest in running studies about different technique investigations in surgeries like electrosurgery, CO_2 laser and radiosurgery; to get the better and fast healing results with less skin disorders or scars. All surgeons are familiar with electrosurgery that was launched as a method of cutting and coagulating tissues. Modern electrosurgical apparatus are able to produce wave forms and incorporate safety features. According to Niamtu (2005) electrosurgery cause significant lateral tissue damage which is generated during incising and coagulating. Radiosurgical developed instrument is used for cutting and coagulating the tissues simultaneously without crushing the cells via alternative electricity with high frequency current and perform more delicate surgeries with a larger margin of safety for the patient (Sperli, 1998). In studies conducted by Bridenstine (1998), and Brown (2000), it was found out that radio surgery by means of radio wave's vibration energy, has less tissue damages than electro surgery. Radio surgery is useful in variety of surgeries in ventral cavity like liver lobotomy, uterine horn resection, sampling of soft tissues, and etc. (Hirota *et al.*, 2005; Risselada *et al*., 2010). There is also a research that compared five different devices: electro surgery, radio surgery, CO2 laser, scalpel and biopsy punches in four adult dog skins for measuring the char rate in biopsy site. Char was not observed around the biopsy's site by scalpel or biopsy punches and there was lesser char by radio frequent electrode than the electro surgery (Silverman *et al.*, 2007). Limitations of experimental studies in human being according to the ethics and unwillingness of surgeons to use electrocutes suggest study on more details to compare the advantages of radio surgery over the normal cutting by scalpel blade and to find out the difference of the healing process between these methods. So, we conducted the present study and used light

microscope to observe tissue damages and healing process improvements in 12 mice, in 21 days after cutting the ventral skin to help choosing the best technique after all.

MATERIALS AND METHODS

An experimental study was designed in that twelve female mice of same age (from Pasture Institute) were randomly selected. As we want to compare scalpel blade surgery and radio surgery, so two different incisions were made in one mouse. Therefore they assigned into three groups consisting four mice with two incisions on each one. They kept for one week in their cage as adaptive period. The upper incision made by scalpel blade were named as group 1 and the lower incision made by radio surgery were named as group 2.

Immobilization of the mice was performed by a cotton soaked in Ether solution (Merk, Germany) the each mouse anesthetized with intramuscular injection of 60 mg/kg ketamine hydrochloride (Alfasan, Netherland) and 10 mg/kg xylazine hydrochloride (Alfasan, Netherland). After the ventral abdominal skin was shaved with povidine iodine solution (Behvazan Lab, Iran), two 3 cm long - incision with 4 cm distance apart were made transversally deep to the full skin thickness, one by number 15 scalpel blade and the second one by radio frequent instrument (Radio Surgery Equipment, Ellman, USA). Tangstan needle electrode by purred waves of 4 MHz and 100 watt electricity power were used for cutting and coagulating combination. Two mice were euthanized and considered for immediate sample as 0-day sample and the other incisions were re- approximated with non-absorbable suture (number4-0 silk, merslene-eticon suture, USA). Wounds were sutured by interrupted simple pattern and dressed by Nitroforazone topical ointment. Mice were kept separately in individual cages and fed by normal commercial diet (pasture Institute, Iran). They had 12 hours light and 12 hours darkness. Skin samples (3x1 cm size) were collected from the incision sites from the two randomly selected mice at 0, 7, 14 and 21 days post-incision and fixed in 10 percent buffered (with phosphate mono and di basic) formalin. These skin samples were processed according to standard histological techniques and embedded in paraffin. Sections of 5 µm were cut and stained by H and E staining method for light microscopic study. Changes in the incision area were compared between scalpel blade and radio surgery incision techniques. Data were collected on the healing process including re-epithelialization, inflammatory reaction, collagen regeneration, granulation tissue composition and measurement of the epidermis, dermis, collagen thickness, inflammatory cells and granulation tissue region, on date of 0,7,14, and 21 days after incision. The measurements were done by Photoshop CS5, the mean of results simply compared between two methods.

RESULTS

To compare scalpel blade and radio surgical equipment cutting edge in detail, we observed tissue damage rate and the healing process improvements of both methods in ventral skin samples. Data were collected in days 0, 7, 14, and 21 after procedure. Epidermis, dermis, inflammatory cell layer, collagen layer, number of hair follicles in each 50 µm² were measured by Photoshop Cs5 software. We compared the healing process between two incisions and the results are explained below step by step. Inflammatory cells after cutting with the scalpel cause delay in healing and thickness of fibrosis tissue in the cutting site after 21 days, versus necrosis by cicatrical process in the cutting edge with radio frequency helps rapid repair and less scar tissue that is observable after 14 days of cutting. The results showed that using radio surgical instruments cause less tissue damage and more rapid healing process in comparison the scalpel blade.

0-day sample: Scalpel blade incisions (group 1) had straight edge without necrosis. Connective tissues and muscles in dermis were situated in normal place. In group 2, samples incised by radio frequent instrument showed coagulative necrosis deep up to 63 µm. Dermis' measure was about 69 µm (Fig.1, 2).

7-day sample: In group 1 samples, there were fibroblasts and inflammatory cells under the epidermis. Thickness of epidermis increased in comparison to the skin in 0-day sample in the same group from about 4 to 15 µm. Dermis was thickened up to 174 µm while it was about 76µm in first day of sampling in the same group and it is because of some degrees of hyperplasia in it around incision margins. In group two, epidermis which were necrotized in first day by radio surgical instrument, was repaired, its thickness was more than 0-day sample (from 5µm to 16µm) and barely the cutting edge was recognizable. Granulation tissue in dermis composed of vessels, fibroblasts, macrophages and new generated collagen fibers. Granulation tissue was about 123µm. Some degrees of hyperplasia in dermis layers were observed and its thickness was 85 µm (Fig. 3, 4)

14-day sample: In group one sample, there were some wound contractions to inside the incision margin was observable; collagens in dermis were presents with scattered macrophages and Skin accessories like hair follicles, they were 1.4 hair follicle in each 50µm². Dermis was thinner in comparison to the first day in the same group and it was about 25µm. In group two, epidermis thickness was back to normal like in first day of sampling and it was about 7µm. Dermis regeneration was accomplished by hair follicles; sebaceous gland and collagens composition, there were 2.2 hair follicles in each 50µm². Collagen layer in dermis was about 60µm and in parallel order (Fig. 5, 6).

21-day sample: Skins were completely repaired and the incision site could hardly be recognized.

DISCUSSION

The purpose of the study was to compare difference of the healing process between scalpel blade and radio surgical incisions. The results suggest that the healing of the incision by radio frequency was processed sooner than scalpel blade incision. This is possibly due to several interfering factors such as more inflammatory cell's presence in scalpel blade incision site in the first week. Second, cicatricle process in radio surgical incision could help rapid wound healing, which has also been observed in the previous study in that using scalpel blade need

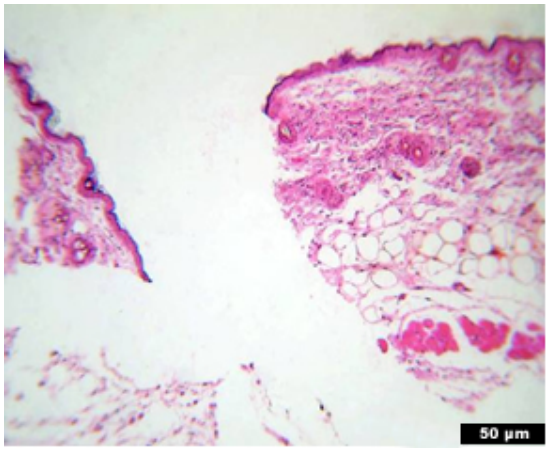

Fig. 1: Mouse ventral skin sample in day 0, cutting by scalpel, straight cutting edge without necrosis (H&E, X10).

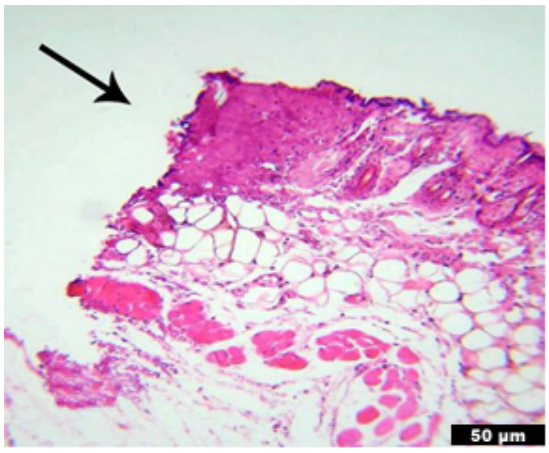

Fig. 2: Mice ventral skin sample in day 0, cutting by radio frequency, necrosis (arrow) in around cutting edge in whole skin thickness, deep up to 63µm (H&E, X10).

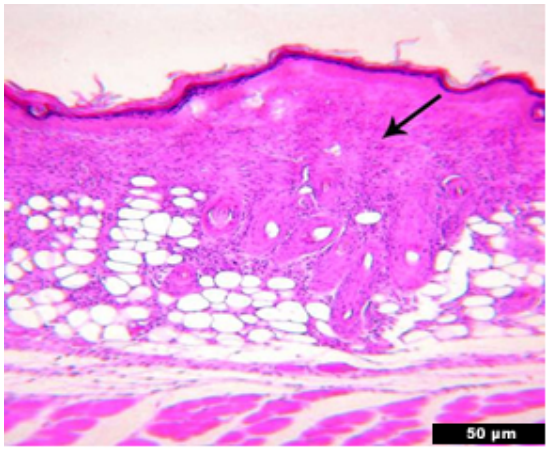

Fig. 3: Mice ventral skin sample in day 7, cutting by scalpel, more inflammatory cells (arrow) in dermis with thickness of 175µm (H&E, X10).

longer time especially for repairing, but radio surgery by haemostatic characteristic, delicate cutting, variety of electrode, and less tissue damages was better technique and helps faster healing process (Saidi *et al.*, 1994). And finally, the wound contraction, granulation tissue formation

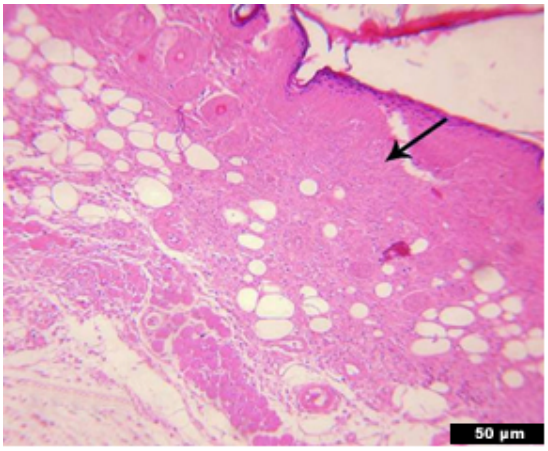

Fig. 4: Mice ventral skin sample in day 7, cutting by radio frequency, epidermis: 16µm and dermis: 85µm with more granulation tissue layer with inflammatory cells (arrow) was 123µm (H&E, X10).

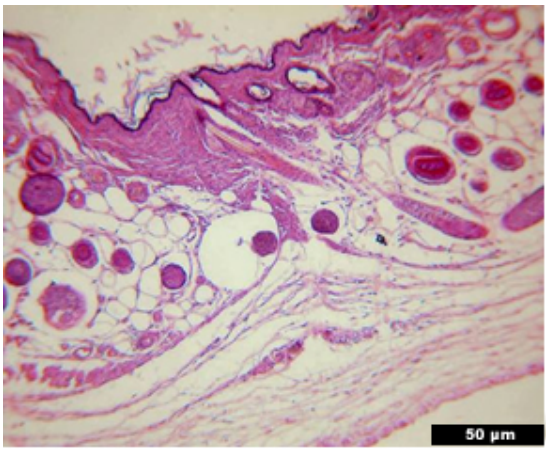

Fig. 5: Mouse ventral skin sample in day 14, cutting by scalpel, epidermis thickness: 6.48µm, wound contraction to the inside and thinner dermis: 25µm (H&E, X10).

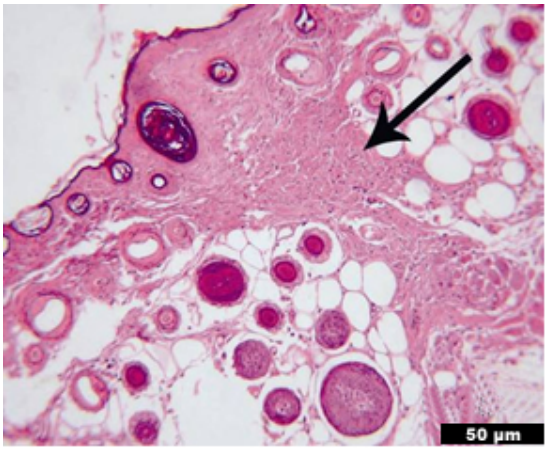

Fig. 6: Mouse ventral skin sample in day 14, cutting by radio frequency, epithelial complete formation and back to normal thickness about 7µm, Dermis: 60µm with 2.2 hair follicles in each 50µm² and more collagen in parallel order(arrow) (H&E, X10).

cellular proliferation, and skin reconstruction would be in better in radio surgery which were confirmed in other investigation of healing and repairing in diabetic rats (Li *et al.*, 2011).

The data are in agreement with Niamtu (2005) histological studies that showed tissue damages by radio surgery were lesser than ordinary scalpel blade like cold blade. Our findings support those of Brown *et al.* (2000), Niamtu (2005), Silverman *et al.* (2007) and Li *et al.* (2011), in decreasing the wound contraction, better collagen formation and less following problems after surgery with radiofrequency in comparison with the scalpel blades. Collagen alignments and rapid maturity have also observed in many researches that radio frequency via less heating and collagen making stimulation in peelings, acne treatments and decreasing the wrinkles in skin could be better choice (Acland *et al.*, 2001; Sadick *et al*., 2011). Our observations provide more evidence for the other study like Alvarez *et al.* (2008) who showed radio frequency applicators helped collagen making stimulation, increasing the elastic fibers and mucopolysaccharides in guinea pigs' skin.

However, the results are in disagreement with one study on rat human modeling showed that distortion after glossectomy by radio surgery was lesser than electro surgery, although cutting with scalpel yet was the best (Kakarala *et al.*, 2010). Our findings also provide no evidence for those of Pollinger *et al.* (2003), Loh *et al.* (2009) which showed no significant differences between scalpel and radio frequent electrode healing process in pigs or mentioned scalpel as a more accurate device in this regard.

The difference may be due to the fact that the size of scar is dependent on the surgeon skill, site and kind of surgery which is important to choose the device and each one has own benefits, for example in 50 patient aged over 10 years old that need tonsillectomy, electro surgery was recognized with better result with less healing problem (Aksoy *et al.*, 2010), but in treatment of tonsil hypertrophy removing a very delicate layer of tonsil by means of radio frequency, considering to less tissue damage it has, noticed more desirable (Plant, 2002)

One interesting finding was that thermal affected region by radio surgery in 0-day skin sample was about 63µm, however, it was a bit different from findings of Bridenstine *et al.* (1998) that reported this region was about 75 µm. This may be defined by the fact that radio surgery make healing process in shorter time and fewer scars will be left by few heating in cutting edge, so radio frequent instrument could be better than the other new methods especially in beauty surgery (Brown, 2000; Niamtu, 2005).

One limitation to this study was that we did not have the possibility of testing different modern incision techniques at the same time. We recommend further studies to investigate the differences in more detailed especially by using the electron microscope.

Conclusions: Our data support using radio frequent instruments over scalpel blade in sites that surgeons need less scar and rapid tissue repairmen. Furthermore, variety of electrodes, portable system, the least dangers comparing to the Laser in cutting, and returning the investments on this equipment considering to no need to buy scalpel blades, make this technology very useful and valuable for the surgeons. Our data also show some benefits, however it needs more detailed researches by new molecular methods in tracing the related molecules in healing process.

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