INFRARED POLARIZED LIGHT FACILITATES AND IMPROVES THE QUALITY OF SKIN WOUND HEALING IN THE RAT MODEL. (EXPERIMENTAL STUDY)

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Abstract: *The aim of the present study was to investigate the effects of polarized-infrared light on secondary skin wound healing of full thickness skin wounds in a rat model. Full thickness skin wounds sized 2 by 2 cm were surgically inflicted in two groups of male Wistar rats, 20 animals each. In the first group (experimental group - EG) the animals were exposed 7 min daily to polarized-infrared light produced by a BIOPTRON device. In the second group (control group - CG), the animals were subjected to the same procedure, but with the device not activated. Mice were sacrificed on 0, 5th, 10th, 15th and 20th day, following the infliction of skin defect. Size and healing process of each wound were recorded and evaluated by means of planimetry and histological examination. Skin biopsies were taken from euthanized rats and histological examinations were prepared.*

According to our findings with the planimetry evaluation, there was acceleration of the healing process in experimental group, whereas an improvement of healing process was identified at each time of histological examination, compared to the control group.

Key words: *polarized-infrared light, wound healing, neovascularization, re-epithelialization.*

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ΤΟ ΥΠΕΡΥΘΡΟ ΠΟΛΩΜΕΝΟ ΦΩΣ ΔΙΕΥΚΟΛΥΝΕΙ ΚΑΙ ΒΕΛΤΙΩΝΕΙ ΤΗΝ ΠΟΙΟΤΗΤΑ ΕΠΟΥΛΩΣΗΣ ΤΩΝ ΤΡΑΥΜΑΤΩΝ ΣΤΑ ΠΟΝΤΙΚΙΑ. (ΠΕΙΡΑΜΑΤΙΚΗ ΜΕΛΕΤΗ)

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Περίληψη: *Σκοπός της παρούσης μελέτης ήταν να εξετάσει τη δράση του πολωμένου φωτός, της υπέρυθρης ακτινοβολίας, στη διαδικασία επούλωσης χειρουργικού τραύματος κατά δεύτερο σκοπό σε επίμυες Wistar. Στην ραχιαία επιφάνεια του σώματος των ζώων δημιουργήθηκε χειρουργικό τραύμα διαστάσεων 2x2 cm^2. Για την επιτυχία του σκοπού της μελέτης χρησιμοποιήθηκαν δύο ομάδες από 20 αρένες επίμυες. Οι 20 επιμύες της πρώτης ομάδας απετέλεσαν την πειραματική ομάδα και τα τραύματα τους υποβάλλονταν σε 7 λεπτών καθημερινή ακτινοβόλιση με πολωμένο υπέρυθρο φως παραγόμενο από μηχάνημα εκπομπής BIOPTRON. Εις την δεύτερη ομάδα ελέγχου τα ζώα υποβαλλόταν εις την ίδια διαδικασία αλλά το μηχάνημα ήταν απενεργοποιημένο. Οι επιμύες*

θυσιάζονταν την 0, $5^η$, $10^η$, $15^η$, και $20^η$ ημέρα, ακολουθούσε αφαίρεση του δέρματος της τραυματισμένης περιοχής. Οι διαστάσεις του τραύματος και η διαδικασία επούλωσης καθοριζόταν με τη μέθοδο της πλανιμετρίας και με ιστολογικές εξετάσεις. Βιοψίες πάρθηκαν από τους επιμύες που είχαν θανατωθεί με ευθανασία και έγιναν τα παρασκευάσματα για τις εξετάσεις.
Σύμφωνα με τα αποτελέσματα της πλανιμετρίας, υπήρχε επιτάχυνση της διαδικασίας επούλωσης στην πειραματική ομάδα, ενώ αύξηση παρατηρήθηκε στην διαδικασία επούλωσης και από τις ιστολογικές εξετάσεις, συγκριτικά με την ομάδα ελέγχου.
Λέξεις κλειδιά: *υπέρυθρο πολωμένο φως, επούλωση τραύματος, ανάπλαση αγγείων, νέο-επιθηλιοποίηση*

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INTRODUCTION

The Hungarian scientist Mester[1,2] is one of the pioneers with large experimental and clinical experience in the use of biostimulating effect of laser beams. A hypothetical model based on experimental facts to explain this effect was proposed. The substance of that model showed that the polarized light reorders the polar heads of the lipid bilayer (being near transition phase) in the cell membrane. The change of the polar heads of the lipid bilayer in the cell membrane has influenced all processes closely connected with it. Another interesting finding pointed out that while the incoherent irradiation of a thermal light source (630 nm) was ineffective, the result of irradiation by polarized light showed about equal effectiveness (80%) of helium neon (HeNe) polarized laser light.[3,4] This phenomenon indicates that biostimulasion might also be produced by polarized thermal light source. Mester and Fenyo[1-4] similarly suggested that a source of incoherent light emitting polarized rays would induce biostimulative effects in living cells similar to low level lasers. This means that lamps emitting polarized light and low-laser light, when used as wound treatment, both cause the phenomenon of biostimulation.

Several experiments in wounds with polarized light showed a stimulating and healing effect.[1] Kubasova[5] used human embryo fibroblast cell cultures to examine the effect of HeNe laser polarized light on the cell membrane. This study showed that 4j/cm^2 was effective for irradiation and the duration of exposure was 7 mins.

Sommer and Franke[6] supported that lower energy densities promote a significant biostimulation in human tissue. They introduced a new laser beam distribution system for medical application, i.e. low energy densities applied with high–energy lasers in combination with laser beam diverging lenses. They found that this system is effective for biostimulating human tissue and also is of low cost. The lamp emitting polarized light used in the present study was also chosen because we assumed that it has a similarly biostimulating effect with low laser polarized light.

Mester et al.[1-2] positive view of biostimulation was a motivation for the present study, in which the aim was to evaluate what is the effect of polarized infrared light on secondary skin wound healing.

MATERIALS AND METHODS

Forty male Wistar rats, 4 months old and weighting 200 ± 30gr, were used and cared for according to Greek and European guidelines regulating animal research. Animals were acclimated for a period of 3 days, during which they were examined for any signs of disease. Throughout the entire study period, the animals were housed individually, in order to avoid cannibalistic behavior, in plexiglas cages covered with stainless steel lids. Their diet contained dried pellets and tap water was supplied to them. The animals were kept in a room under stable conditions of temperature (22 ^{o} C) and humidity (between 30%-70%), and the light cycle was 12/12-hours light/dark schedule. On day 0, anesthesia was induced by intraperitoneal injection of a mixture of Ketamine and Midazolamin (3.5 mg and 7mg respectively per kg animal's body weight). The whole surgical procedure was conducted under aseptic conditions. A square shaped area measuring 2 by 2 cm was marked on the dorsum of each animal, followed by a block excision of the skin and underlying panniculus carnosus. The animals were randomized in two groups of 20 rats each. In the first group, those receiving treatment were called Experimental Croup (EG) and those not receiving treatment, Control Group (CG).

The treatment consisted of a daily 7 min therapy session with polarized infrared light. This started on day 0 and finished on day 20. During the experiment, one rat at a time, was placed and immobilized in a special constructed wooden box with no metallic components, in order to avoid any interference with the polarized light. The device was vertically centered over the box at a constant distance of 10 cm

from the wound surface, so that the wound area was received the whole degree of polarization. The experiment was conducted using the non-invasive and non-thermal Bioptron Compact III[7] modified with a filter wratten number 25 (which absorbs the yellow light) as a result, the remaining light was infrared wavelength of 700-3400nm. Energies delivered were typically 2.4 joule/cm^2 per minute, degree of polarization >95%, wavelength 480-3400nm, power density 40 mW/cm2, using a 20W[7].

After the experiment the rat was returned to its cage and housed separately without using any dressing or antibiotics on the wound.

On the 0, 5th, 10th and 15th day after wound creation, four rats of each group were euthanized. Wound surface area was measured by tracing the border of the wounds on transparent film (table 1). The area from these tracings was measured with a polar planimeter (model N^o 317 E, Manufacturer of HAFF planimeters: W-Germany. The precision of this instrument is within 1mm^2).[8]

Skin biopsies were taken from mice that were sacrificed on the 5th, 10th, 15th and 20th day after skin infliction. The tissue specimens were fixed in 10% formalin solution, and both wound and surrounding healthy tissue were harvested for histological examination. All specimens were paraffin-embedded, cut in 5mm thick blocks, perpendicularly to the skin surface, including the whole thickness of the skin wound.

The slides obtained were stained with Heamatoxylin-Eosin (HE), Vascular Endothelial Growth Factor Ab-1 (VEGF Ab-1, dilution 1:100, LAB VISION), α-Smooth Muscle Actin Ab-1 (α-SMA Ab-1, dilution 1:200, LAB VISION), Vimentin Ab-2 (Vim Ab-2, dilution 1:400, LAB VISION) and Van Gieson stain (VG).

The VEGF LAB VISION antigen is specific for the Wistar rat. This antibody is a homodimeric, disulfide-linked glycoprotein involved in the angiogenesis. Its permeability to the vascular endothelium is specific and act as diffusible agents especially in the smaller isoforms. The α-SMA antibody stained the smooth muscle cells in the newly formed vessel wall and it was found mainly intracytoplasmic. Vimentin was the main intermediate filament protein in mesenchymal cells and was therefore found in great quantity in the vascular smooth muscle cells and its staining pattern was cytoplasmic. Van Gieson stain highlighted the collagen bundles formed by the fibroblasts and it was found in great quantity in older granulation tissue, starting from the margins of the wound during the first days and finally spreading through out the wound.[9,10,11]

We scored and evaluated 4 different parameters of wound healing:

a) Epithelization
b) Inflammation
c) Newly-formed vessels
d) Collagen formation

We blindly scored the microscopic slides for the two groups in question (EG and CG) in order to avoid any misguiding influence.

Exact measurements of the degree of epithelization (both in the experimental and control wound groups, EG and CG) were taken. This was done by microscopically measuring the entire wound area and also the surface area at the margins of the wound, where epithelization had already started.

Epithelization scoring is shown below:[12]

0	Indicates no epithelization
1	Indicates no epithelization
2	Indicates ≤40% of complete epithelization
3	Indicates ≤60% of complete epithelization
4	Indicates ≤ 80% of complete epithelization
5	Complete epithelization

Following this, we evaluated the degree of inflammation of the wound. In our study no acute elements of inflammation where observed in the tissue biopsies since specimens were received from the day 5. Polymorphonuclear granulocytes (PMNGs) where seen in the blood

clot above the wound since PMNGs help clean the wound from necrobiotic material during the first days.

The amount of newly formed vessels were also estimated and scored subjectively. We revealed the endothelial cell lining of the newly formed vessels with VEGF stain. They were scored from 0-3 according to their density in the granulation tissue.

Finally, we evaluated the intensity of the collagen bundles, measured their distance from the surface of the wound and the degree of collagen formation from the fibroblasts with the Van Gieson stain. Wounds which had collagen in the deepest area (>0.6mm from the surface of the wound) stained only weakly and scored 1. Score 2 was given to those wounds with collagen which stained moderately and found up to the mid-portion of the wound (app. 0.5mm), and score 3 was given to those wounds with collagen found in the whole thickness of the wound that stained intensely with the Van Gieson stain.

All of the above listed parameters were scored as follows:

0 = None
1 = Mild
2 = Moderate
3 = Severe

STATISTICAL ANALYSIS

The t-test and one way analysis of variance were used to evaluate the significance of differences within and between groups, accepting 5% ($p < 0.05$) as the level of significance. The significance of the results obtained is supported by histological evaluations.

RESULTS

Throughout the entire experiment, all rats in both groups remained healthy. All wounds sites showed the normal wound healing process with no signs of infection or purulent discharge. In the experiment the observed process in both groups is healing by secondary intention.We observed that the experimental group developed earlier formation of newly formed capillaries and in larger quantity (particularly during the first 5 days) compared to the control group. Another finding was that after the 15th day there was a decrease in neovascularization and also the capillaries were more mature and well established in EG group.

Referring to the planimetric evaluation, although we observed acceleration of wound healing in the EG, in the overall experiment there was no statistically significant differences between the two groups (p=0.508). We observed though significant differences between the two groups on 10th and 15th day (p=0.017 and p=0.036 respectively). The differences between those rates are clearly represented by bars given in Figure 1.

Histologically the following findings were observed:

Day 5: The histological results showed that in the control group (CG) the epithelization and the produced collagen was measured and scored 1.Underneath the blood clot there was loose granulation tissue with few stimulated fibroblasts producing collagen around the margins of the wounds. The newly formed endothelial cells were scored 2 as only small and moderately dense capillaries were seen. (Figure 2A)

In the experimental group (EG) a slight epithelization was observed and scored 1. The presence of several fibroblasts was evident and some collagen bundles had already started to form at the base of the wound, 0.5mm from the surface, scored also 1. The density of the newly formed capillaries was noted and stained more intensely in the EG than in the CG with VEGF, score 3 (Table 2 and Figure.2B). This finding was also confirmed with Vim and a-SMA which stained the pre- and post-capillary vascular smooth muscle cells.

Day 10: The wound healing in the CG showed an epithelization which was measured and scored 1 whilst in the EG scored 3. The newly formed capillary network, scored 2 both in the CG and the EG as it was demonstrated by VEGF, Vim and a-SMA Also there was an increase in the formation of collagen by fibroblasts at the

base of the wound, in the CG scored 1 and in the EG scored 2, (Table 2 and Fig.3A,B).

Day 15: The epithelization was still in an incomplete fashion and was measured and scored 3 in CG, whilst in the EG measured and scored 4. The same moderate amount of fibroblasts with abundant bundles of collagen were found in the CG as well as in the EG on the 15th day, scored 2. The capillary network was more mature than on the 10th day both in the CG and the EG but the score was the same for both groups, 1, as no new capillaries were formed (Table 2 and Fig. 4A,B,C,D).

Day 20: In the final day of our experiment, similar findings were recorded, in both groups (Table 2). Although quantitive results are similar, in the EG better epithelization and more mature fibroblasts creating a denser collagen deposit parallel to the surface was observed thus creating a better quality of wound healing (Figure 5A,B).

DISCUSSION

A number of studies have assessed the healing process of wounds treated by light using different wavelengths (i.e. low level laser light) but controversies appeared between them as some were unable to show a beneficial effect.[13,14] Controversies might be accounted for the different criteria and from the variety of appearance of the wounds. Some other studies indicated that light therapy could be of great benefit in the treatment of chronic wounds although the design of these studies was different from the present one.[5, 6]

In our work, although, we did not observe any statistically significant differences in the overall healing process between the two groups, the results showed that most of the rats of EG had nearly completed the wound healing process on the 15th day compared to the CG which had complete healing on the 20th day. Thus acceleration of wound healing with a non invasive method, such as infrared polarized light, may be important in reducing bacteria accumulation, stimulating growth factor and reducing early inflammation, creating an appropriate environment to facilitate tissue regeneration.[15,16]

Danos[17] et al findings are very similar to those of the present study. They used cultural human keratinocytes, endothelial cells and fibroblasts exposing them to the infrared light and they concluded that the infrared irradiation potentially enhances the wound healing process, supported by its biostimulatory effects.

Madenica,[18] using the polarized polychromatic non-coherent light for venous leg ulcerations and from their immunochistological analysis, showed that all tissue specimens revealed significant histologic changes after three weeks of treatment. Sections stained by Masson' trichrome showed significant proliferation of fibrotic tissue. All of these sections demonstrated extensive neo-angiogenesis and increased vascular density.

Smith[19] suggested that the infrared spectrum initiates the response at the membrane level, through photo physical effects on Ca^{++} channels.[19]

Whilst Slavin[20] indicated in his study that low power laser light therapy stimulates release of growth factors from irradiated cells. Growth factors stimulate angiogenesis, extra cellular matrix production and degradation.

The main finding from our study after the use of infrared polarized light was the accelerated reduction of wound surface area. This finding was also histologically proven by the faster transformation and maturation of myofibroblasts, forming better quality of collagen bundles parallel to the surface and faster re-epithelialization of the wound.

CONCLUSION

In conclusion, the findings of the present study indicated that infrared polarized light seem to facilitate and improve the quality of skin wound healing in the rat model. Nevertheless, further studies are required to define the optimal spectrum of which will ensure a better and faster pattern of wound healing.

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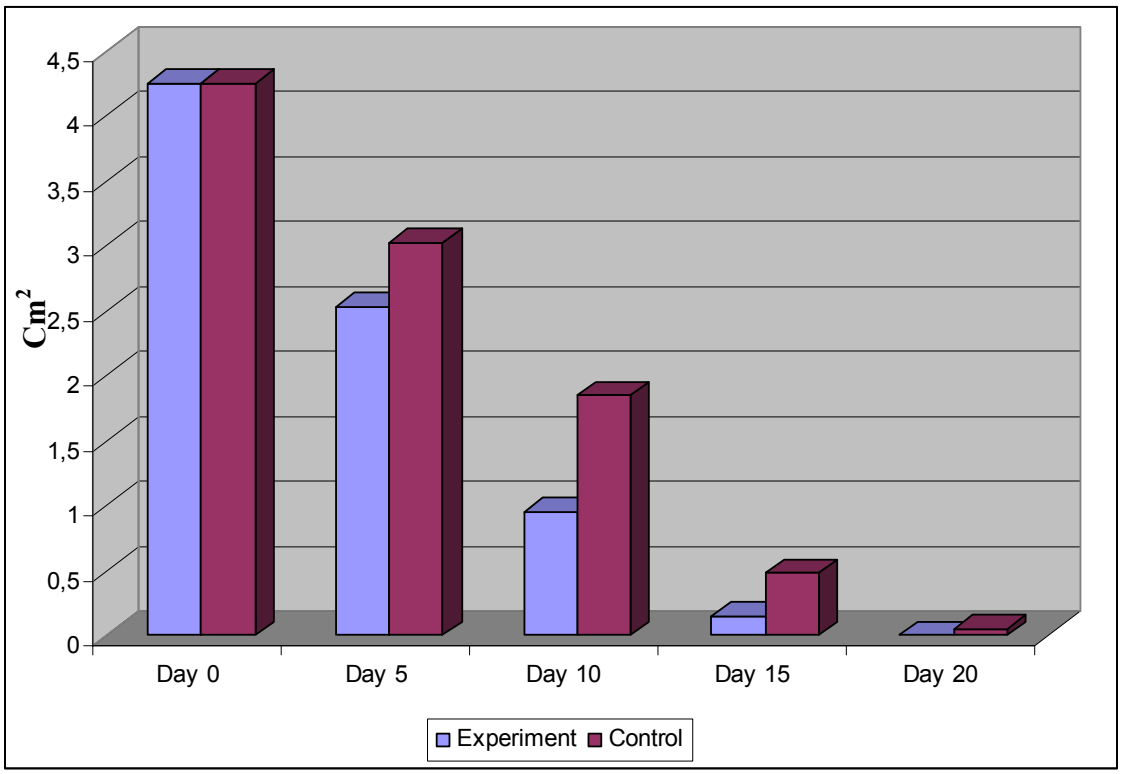

Figure 1: Wound healing slopes inclination. Note that wound healing in experiment group was already completed in most of animals at 15th day of exposure in contrast with control group that completed in 20nd day. But healing rate was not statistically significant between two groups.

	Exp.Group			Control Group				
Day	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**t**	**Sig. (p-value)**
0	4	4.25	0.2664	4	4.25	0.2664	0,0	1.000
5	4	2.53	0.3211	4	3.03	0.2860	-2.325	0.059
10	4	0.95	0.4268	4	1.85	0.2008	-3.816	0.017
15	4	0.15	0.1151	4	0.48	0.1984	-2.876	0.036
20	4	0.0	0.0000	4	0.05	0.0150	-1.667	0.194

Table 1: *Mean and SD of the reduction in surface area (cm^2) between measurements in both groups. Wound healing surface planimeter analysis. 4.25 cm^2 is the mean range of wound surface, in all animals which have been created after surgical excision. (Due to different tensile strength of skins, or different skin reactions after anaesthetic medication, or surgical manuver in both groups of animals).*

Table 2: *Scoring of the Epithelization, Inflammation, Neovascularization and Collagen formation tissue in the EG and CC groups*

Experimental Group				
Day	**Epith. Score**	**Inflam.**	**Neo-Vasc/tion Score**	**Collagen score**
5	1 (1/18mm)	Negative	3	1 (0.5mm)
10	3 (4/8mm)	Negative	2	2 (<0.5mm)
15	4 (3/5mm)	Negative	1	2 (<0.5mm)
20	5 (complete)	Negative	1	3

Control Group				
Day	**Epith. Score**	**Inflam.**	**Neo-Vasc/tion Score**	**Collagen score**
5	1 (1.5/16mm)	Negative	2	1 (>0.5mm)
10	1 (1/18mm)	Negative	2	1 (>0.5mm)
15	3 (4/6mm)	Negative	1	2 (0.5mm)
20	4 (complete)	Negative	1	3

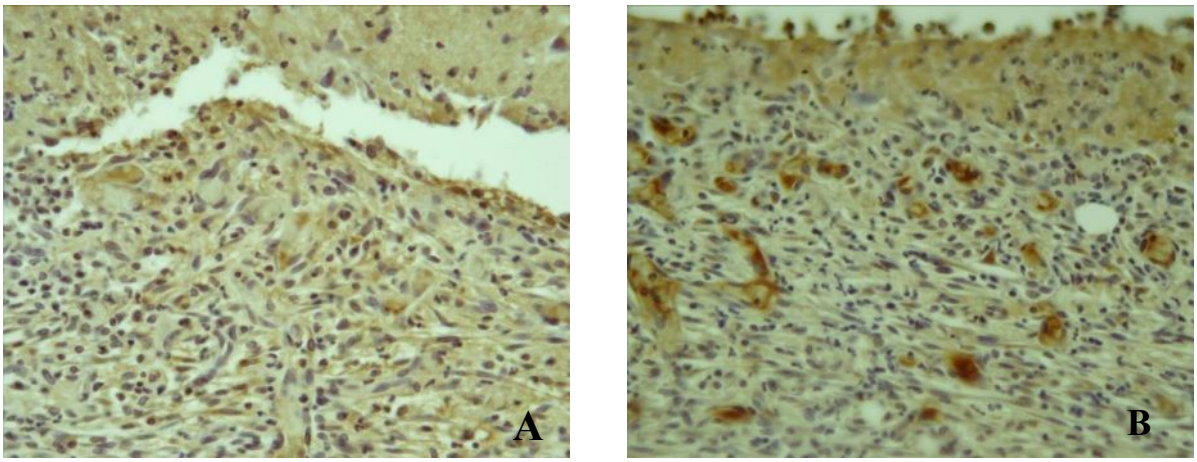

Figure 2

Day 5 :

A) Control Group: immature endothelial cells and newly formed capillary network. (VEGF, 20X).
B) Experimental Group: more mature endothelial cells and better formed capillary network are evidend. (VEGF, 20X).

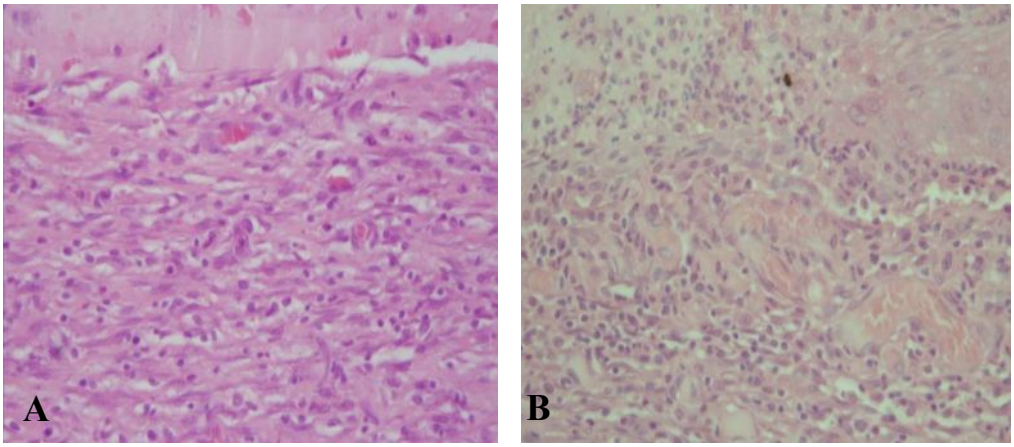

Figure 3

Day 10 :

A) Control Group: decrease in PMNGs and denser connective tissue (H&E, 40x).
B) Experimental Group: slight epithelization, mature capillaries and parallel to the surface fibroblasts (H&E, 40x).

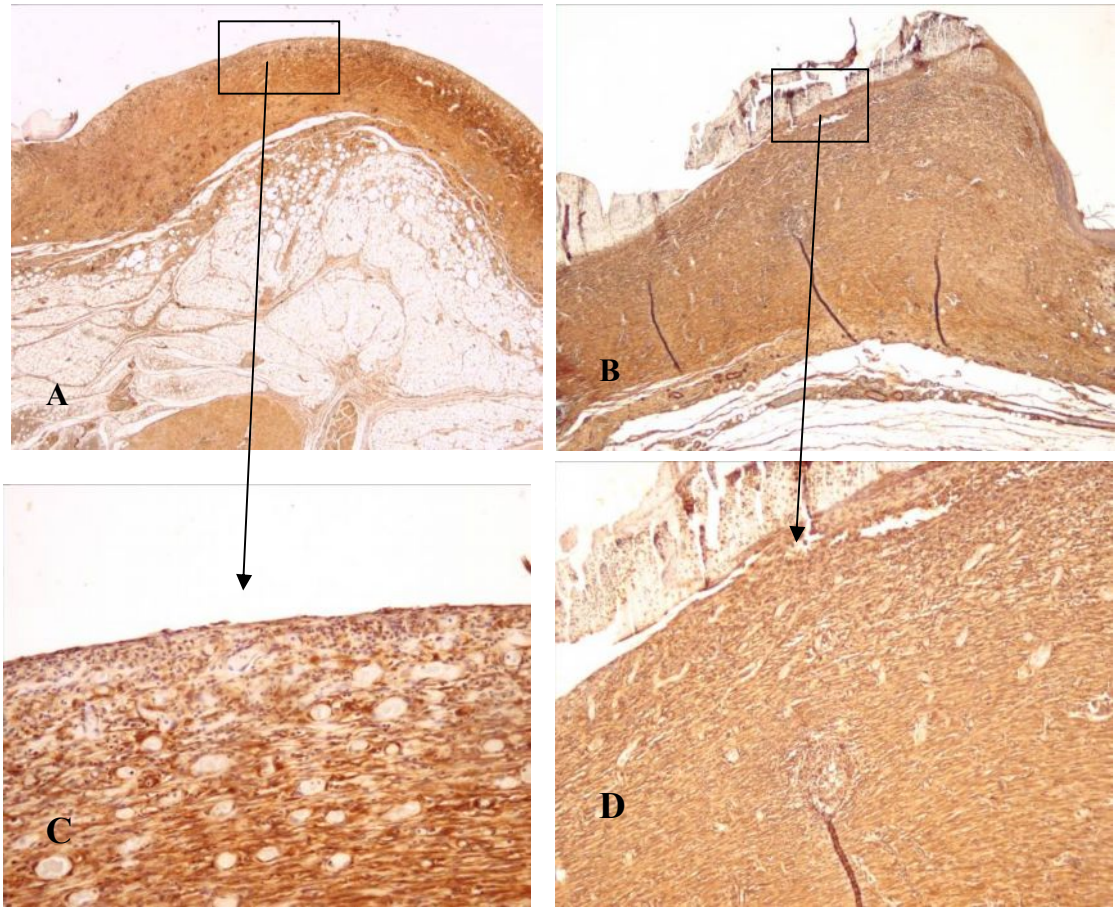

Figure 4

Day 15 :

A) Control Group: slight only epithelization and immature fibroblasts in the granulation tissue (SMA, 2x).
B) Experimental Group: moderate surface epithelization and mature Fibroblasts (SMA, 2x).
C) Control group: higher magnification focusing on our findings (SMA, 20x)
D) Experiment Group (SMA, 20x).

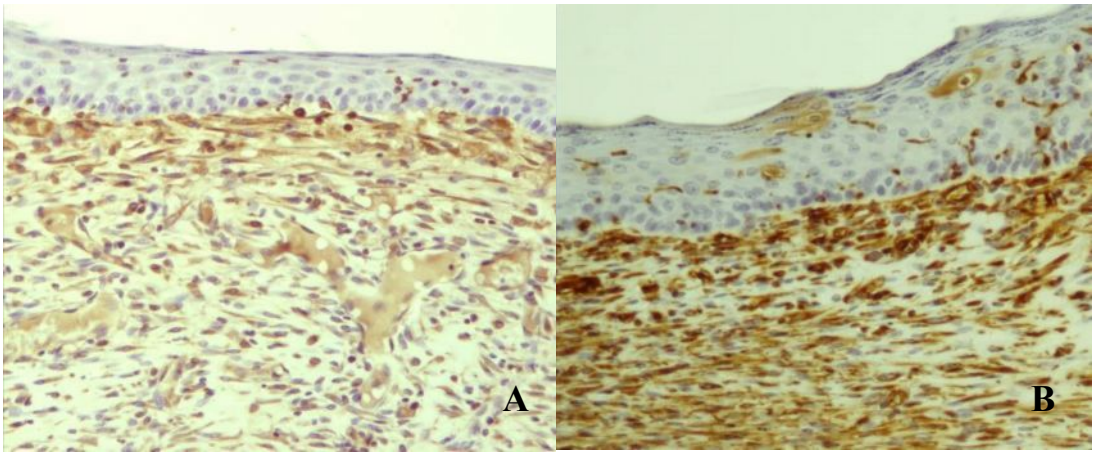

Figure 5

Day 20 :

A) Control Group: complete epithelization and healing (VIM, 20X)
B) Experimental Group: complete epithelization with mature keratinocytes and fibroblasts more parallel to the surface was noted (Vim, 20x).