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Original Article

Comparison of Burn Depth at Different Temperatures on Ex Vivo Human Skin with Standardized Model and Comparison of the Results with Rat Contact Burn Model

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ABSTRACT

Aim: Burns are still an important mortality and morbidity problem all over the world. Clinical studies are limited, owing to ethical concerns and inability to achieve standardization. Therefore, studies are concentrated on experimental models. However, there is still a lot of questions that are awaiting for resolution. On the other hand, the effect of animal models on human skin (HS) is unknown. From the point of view, it was aimed to evaluate the depth of burn on ex vivo HS and to compare the HS results with rats.

Materials and Methods: Following obtaining inform consent, skins of patients that are underwent full thickness healthy skin excision (abdominoplasty), except for experimental purposes, have been included. A total of three different temperatures (60, 80 and 100°C) and two different weight force 0,88kg/cm2 for high and 0,21kg/cm2 for low weight force group were created with standardized apparatus. In all groups healthy dermis-epidermis, burn depth was compared.

Results: No difference was detected between healthy HS depths. At least 10.5±0.7% burn depth was figured out in 60°C-low weight force group and at most in 100°C-high weight force (92.0±2.7) group. Out of 80°C- high pressure group vs 100°C low pressure groups, significant difference was detected in all.

Conclusion: Ex vivo HS could be used as an experimental burn model. It has been shown that standardized depth of burn wound could be achieved on behalf of standardized apparatus. However, the different depth of burn indicates that control of parameters (pressure, time, temperature) is mandatory.

Keywords: Burn, ex vivo, human skin, rat

Introduction

Burns are still a frequent trauma all over the World. According to American Burn Association statistics between 2006-2015, 205033 individuals have been predisposed to burn trauma and 3.3% of them were lost (1,2). Since mid-20th century, owing to experimental or clinical lots of studies, treatment modalities have been improved. However, due to ethics and standardization problems in clinical studies, experimental studies have been mostly preferred for physiopathology and healing procedures (3-9). Therefore, varying number of scalding and contact burn models have been defined (3,10-14). On the other hand, infeasible real-time contact temperature and applied weight force measurements were the weak points of models (3,14). For this reason, in 2016 we designed a standardized contact burn model in which the real-time contact temperature and pressure could be measured (15). Standardized second degree burn can be

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achieved in rats on behalf of custom designed apparatus (15). However, although the created wounds were second degree, the burn percentages were significantly different from each other. Moreover, an experimental model on human skin that is defining the degree of burn, has not been proposed before. From the point of view, it is aimed to answer the questions,

1. What will be the degree of burn depth on account of standardized experimental burn model?
2. The responses and nuances of rat and human skin in a standardized burn model?

Materials and Methods

Study has been conducted following approval of human ethics committee (20/02/2017-80558721/71) of Eskisehir Osmangazi University. Human skins were obtained from the discarded tissue of patients undergoing abdominoplasty, which was assessed as waste material. Before surgery informed consent was obtained from all individuals. Following excision in the operating theatre, skins were wrapped to the fresh frozen plasma(FFP) soaked gauze; transported with a vacuum bottle at +4°C and kept at +4°C to the end of procedure. All experimental steps (burns and biopsy) have been underwent in the same day (0. Day following excision). Custom designed apparatus was used for *ex vivo* HS model (Figure 1). Three temperature groups (60, 80 and 100 °C) and two weight force (WF) groups were designed, while the elapsed time was limited to 10 sec. in all groups. Burns that were achieved with utmost only a contact surface was accepted as low WF group (LWFG) and about 1000 gr of WF was for high WF group (HWFG). However, real time WF force was measured in all burns due to spring loaded design of apparatus. Healthy normal skins of all individuals were used for control group with measuring dermis and epidermis thicknesses (Figure 2).

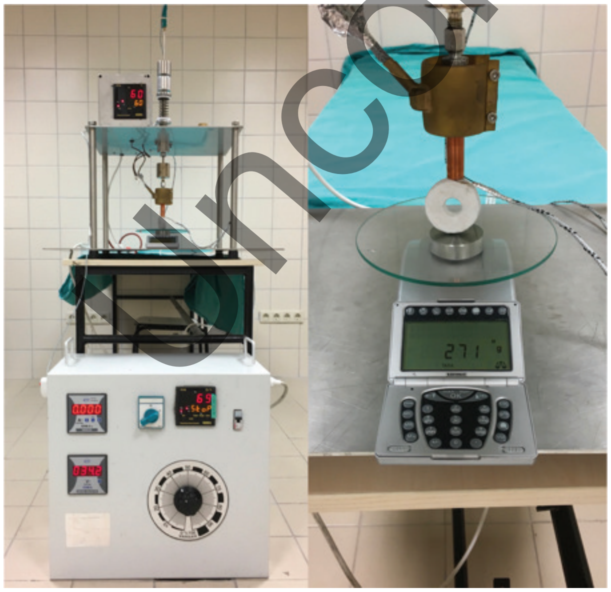

Figure 1. Custom designed apparatus

Group 0: Healthy human skin (control)
Group 1: 60 °C LWFG (G60LWFG)
Group 2: 60 °C HWFG (G60HWFG)
Group 3: 80 °C LWFG (G80LWFG)
Group 4: 80 °C HWFG (G80HWFG)
Group 5: 100 °C LWFG (G100LWFG)
Group 6: 100 °C HWFG (G100HWFG)

In the laboratory, the excised skin samples were cut into strips of 10*5 cm in length and width, and also defatted under the dermal component. The standardized tissue pieces were fixed onto a flat platform to get a perpendicular angle between the burning bar and skin for accurate measuring of WF (Figure 1) with electronic scale. A 10 mm diameter cylindrical burning bar (that has 0.78 cm^2 surface area) was used and at least 7 burns were created in all groups (Figure 3). One hour after the procedure burns were totally excised and specimens were fixed with formaldehyde.

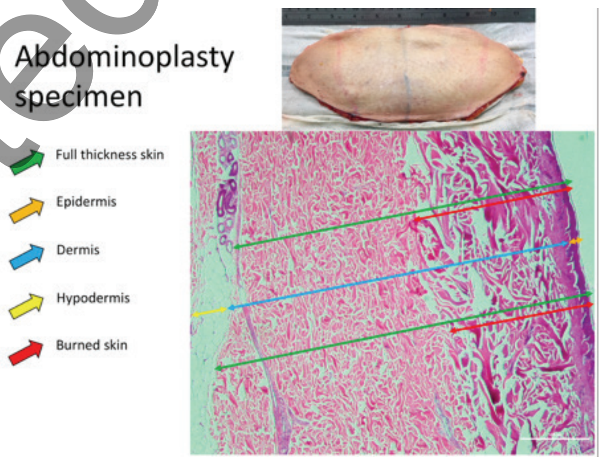

Figure 2. Skin material and histological measurements (Scale = 500 µm)

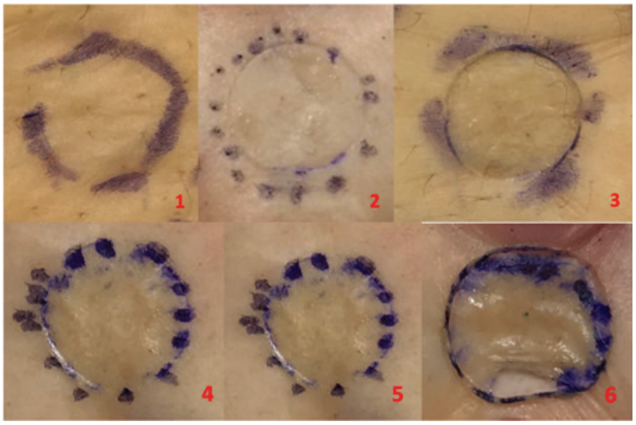

Figure 3. Macroscopic photographs of burns (1:60 °C, low weight force group, 2: 60 °C, high weight force group, 3. 80 °C, low weight force group, 4: 80 °C, high weight force group, 5: 100 °C, low weight force group, 6: 100 °C, high weight force group)

Statistical Analysis

Slices were stained with hematoxylin eosin and examined by a blinded anatomist under light microcopy (Nikon) (Figure

4). Photographs of burns were taken. Skin thicknesses (Dermis, epidermis) and burn depths were measured from three different lines (Figure 4). Mean values and burn ratios (burned/healthy skin) were calculated for each wound with Microsoft Excell sheet. Graphpad Prism 7 software was used for statistical analysis. Normality distribution of data was assessed by Shapiro-Wilk test. Groups were compared using a two-way ANOVA test with post hoc test of Tukey's multiple comparisons. P values less than 0.05 were considered as statistically significant.

Results

Discarded healthy skin of 4 patients were used. Any difference was detected in *ex vivo* healthy HS (dermis and epidermis) thickness of patients (p>0.05). A mean of 0.21 kg/cm^2 WF was applied in LWFG and 0.88 kg/cm^2 in HWFG. And also, neither in LWFG nor in HWFG no difference was detected (p>0,05). The percentage of burns has been given in table I. And out of G80HWFG vs G100LWFG groups all of them have strong significant different depth of burns between each other (p<0.001).

Over and above on *ex vivo* HS, in case of LWF force with a 60 °C first degree burn could be created; superficial second-degree burn could have been achieved if HWF applied in the same temperature. Between superficial and deep second-degree burn was detected in G80LWFG; in the other respect, deep second-degree burn was confirmed in both G80HWFG and G100LWFG groups. Third degree burn has been ascertained in G100HWFG group (Figure 3, 4).

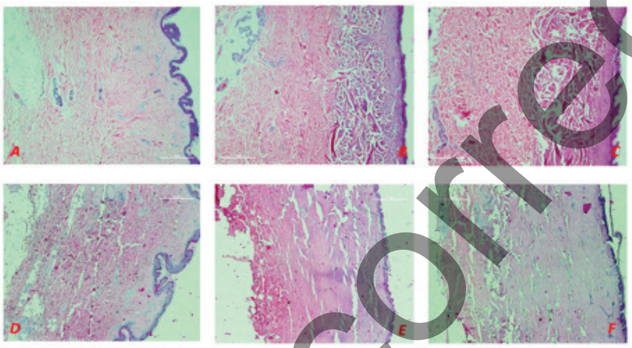

Figure 4. Representative H&E stained sections of groups. (Histological appearance of A: 60 °C low weight force group, B: 80 °C low weight force group, C: 100 °C low weight force group, D: 60 °C high weight force group, E: 80 °C high weight force group, F: 100 °C high weight force group, Scale bar shows 100 microns.)

Table I. Percentages of burn depth in all groups (60: 60°C, 80: 80°C, 100: 100°C

Groups	Burn Depth (%)
60 LWFG	10.5 +0.7
60 HWFG	25.8 +2.4
80 LWFG	52.9 +2.6
80 HWFG	71.1 +2.1
100 LWFG	66.7 +2.1
100 HWFG	92.0 +2.7

LWFG: Low weight force group, HWFG: High weight force group

Discussion

Burns are the most frequent trauma with an incidence of 1.1/100000 all over the world (2). According to trauma statistics, burns are the underlying reason for approximately 5 % of the patients, who are lost due to trauma in the world (1,2). Therefore, studies concerning burns have been going on to evaluate prevention, physiopathology and treatment modalities. Cetin et. al. compared the survival of *ex vivo* HS in fresh frozen plasma(FFP) soaked gauze and saline (16). And found that HS live on longer in FFP more or less about thirty days. Therefore, all study procedures have been undergone in day 0 following excision and we believe that results of the study could best simulate the living HS contact burn wound that has not been reported before. Consequently, *ex vivo* HS and custom designed standardized contact burn model has been used to depict the depth of burn on HS clearly. And also, we believe that the study could provide a basis for a new experimental model on HS. Over and above, study could be helpful for understanding of correlation of percentage, temperature and weight force on HS. Herein, study has been shown that different statistically significant depth of burn, from 10.5% to 92% under the strict control of variables (time, temperature and weight force), on *ex vivo* HS. In comparison to animal model, more superficial burn depths has been figured out on *ex vivo* HS although there was no difference between the steps of experimental model (15). And also, it was realized that out of groups 1 and 6, variable depth of second degree burn was created as if in animal model. Moreover, they all have significantly different percentage of burn wound in each other such as superficial, between deep vs superficial and deep second degree. Thus, might be due to different skin thicknesses or might suggest that human skin is much more resistant than rat's. Finally, we believe that such different percentage of burns might cause variable inflammatory responses and that might impress the healing capacity as well. And hence, it might play an important role on inflammatory and/or healing procedures that should be evaluated.

Conclusion

On behalf of custom designed apparatus standard depth of burn on *ex vivo* HS could have been created. The percentage of burn depth changes according to accurately controlled variables (time, weight force and temperature) during contact burn model on *ex vivo* HS as well. Therefore, they should be strictly under control. Especially for experimental healing models, for standardization of burns, percentage of burns might be a better indicator for classification.

Study Limitations

Ex vivo nature of the study is its limitation that is without blood supply, although skin goes on alive, it is impossible to put forward the immune reactions and treatment studies. However, this model could extend a basis for cell culture studies.

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Conflict of Interest

The authors have no financial interest to declare in relation to the content of this article.

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