# THE ADVANTAGES OF THE APPLICATION OF AMNION MEMBRANE IN THE TREATMENT OF BURNS

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A b s tr a ct: A crucial and important factor for successful treatment of burns is the early covering of the burned area with skin substitutes. The covering of the burn requires material that restores the epidermal function and integrates itself into the process of healing. Biological dressings are the golden standard for the temporary covering of burns. All biological skin substitutes are susceptible to early graft reaction and the only exception is the amnion membrane. The importance of the amnion membrane as a biological dressing for burns amounts to: a barrier to bacterial colonization, hastens the epithelisation, and control of water loss. Amnioplasty is a method of application of amnion membrane on the recipient site.

In this comparative study, 60 patients with dermal and sub-dermal burns were included. Research was made on an examination group of 30 patients with burns where the method of amnioplasty was applied, and for this amnion membrane conserved in

76% alcohol was used. The control group was made up of 30 patients with burns treated conventionally, and standard methods for the local treatment of burns were applied: exposition, occlusive dressing and initial excision with skin grafting. Pathohistological and microbiological analyses of the bioptical material were made. The degree of the burns was determined through a pathohistological analysis of the bioptical material taken the third day, and in some of the subjects where re-epithelialization was determined on the seventh day, the further re-epithelialization was observed clinically. Pathohistological examination enabled discrimination between bacterial colonization and the invasive bacterial infection. Furthermore, the type of bacterial colonization and infection was determined, which was confirmed with microbiological analysis.

The analysis of the results from the microbiological and pathohistological researches of the bioptical material according to the bacterial colonization and infection showed that, although between the examined and the control group there was no statistically important difference, the value of p = 0.067 is close to the statistically important value of p < 0.05. The results of the pathohistological examination of the bioptical material taken the seventh day and analysed according to the re-epithelialization showed that there was a significant difference between the two groups of p < 0.035.

It should be mentioned that, although according to the microbiological examinations of the bioptical material a statistically significant difference was not achieved, clinical significance was achieved. The obtained significance of p < 0.035 compared to the re-epithelialization in both groups approved the application of the method of amnioplasty.

The histological analysis of the bioptical material not only determines the degree of the burns specifically, but facilitates the choice of method for further treatment, observes the speed of the re-epithelialization and plays an important part in the correct diagnosis and the early start of the specific therapy, important in preventing sepsis. The application of amnion membrane as a biological dressing speeds the re-epithelialization and prevents invasive bacterial infection. Pathohistological examination of the burns is recommended to be established as a standard method in clinical practice.

Key words: amnion membrane, burns, biopsy, pathohistological examination.

### Introduction

It has been known for centuries, but never applied, that the adhibition of skin substitutes expedites the healing of skin lesions, including burns. The covering of burns requires material that will restore the epidermal function and will integrate into the process of healing the lesion, taking into consideration that the skin substitute mainly adheres in the granular tissue [1].

Conceptually, skin substitutes are: permanent or temporary; epidermal, dermal or composite; biological or alloplastic.

Biological skin substitutes (dermal allograft, dermal xenograft, human amnion membrane) represent a golden standard for the temporary covering of burns. Biological skin substitutes should have the following qualities [2]: to adhere to the burned surface; to enable low bacterial growth or to prevent subsequent bacterial colonization of the burned surface; to reduce the loss of fluids, microelements and proteins from the burned surface; to enable good permeability of gases and liquids, from the surface of the burn to the tissue around; to decrease the possibility of the creation of scars or keloids in the process of healing; to decrease the pain and to increase the comfort of the patient; and to be easily applicable and removable.

The imperfections of the biological surgical substitutes are: early graft reaction, possible transmission of virus or bacterial infection, and lack of donors.

The allotransplant and xenotransplant have numerous positive qualities, but their use is sometimes limited, especially if tissue banks do not exist. A general disadvantage in the use of the skin substitute is the "early graft reaction" and rejection of the transplant. The amnion membrane is an exception. The amnion membrane does not produce the usual histo-compatible antigens: HLA-A, B or DR. As a result of this the amnion membrane does not indicate immunological reaction after transplantation [3].

The amnion membrane or amnion is the internal layer of the placenta and is in contact with the amnion liquid. Macroscopically the amnion membrane is thin, transparent, resistant, adherent to the chorion from which it can be easily parted with simple traction or with the assistance of a surgical instrument. The normal amnion membrane is 0.02-0.05 mm thin, which is equivalent to 6-8 rows of cells, and has a surface of  $1600 \text{ cm}^2$ .

Histologically, the amnion membrane consists of a thin cubical epithetlium, a thick basal membrane and an avascular stromal matrix [4]. The basal side of the amnion membrane is an ideal kind of substrate which helps the growth of the epithelial cells by prolonging their life and maintaining the clonogenecity [5]. Also, few factors of growth are identified in the amnion membrane. The stromal side of the amnion membrane contains a rare matrixiel component which suppress fibroblast proliferation. This action explains why transplantation of the amnion membrane helps the reduction of the cicatrix during epithelialization.

Actually, the amnion membrane is similar to a "chemical sandwich" which contains antiflammatory and cytostimulatory components [6]. The mitogen factor was separated from the human amnio-chorion which can explain the profuse neovascularisation and the accelerated process of re-epithelialization during the treatment of burns with amnion membrane as a biological skin substitute.

The anti-angiogenesis and anti-inflammatory proteins – interleukin-1, receptoral antagonists, all four TIMPs, collagen IV, laminin, intraleukin-10 – were isolated [7].

Different growth factors have been isolated: the basal fibroblast factor of growth, the transforming  $\beta$  growth factor, the hepatocyte growth factor. The presence of certain protein growth factors can be of benefit to epithelialization after the transplantation of amnion membrane. The high level of EGF, KGF, HGH and bFGF in the amnion membrane with an epithelium, compared with amnion membrane without an epithelium, suggests the epithelial origin of these growth factors [8].

The amnion membrane does not produce the usual histo-compatibile antigens HLA-A or DR. As a result of this the amnion membrane does not induce an immunological reaction after the transplantation, thus taking the primacy as "the ideal" temporary skin substitute.

Amnioplasty is a method of application of amnion membrane to the recipient site. Actually the amnion membrane is a temporary skin substitute, and the recipient sites are the regions with loss or defect of the skin layer. The future of the transplantation of amnion membrane in a number of medical subspecialties can be seen in the application of human acellular amnion membrane (HAAM). HAAM is prepared with chemical detergent-enzyme extraction. This proceedure eliminates the epithelial cells – the soluble elements – makes for good preservation of the tissue matrix and preserves the reticular structures. The human acellular amnion membrane is an ideal biological membrane for tissue engineering [9]. The amnion membrane as a biological skin dressing, when treating burns, is closest to the "ideal" temporary skin substitute. The amnion membrane has these qualities [10]: transparency; semipermeability; elasticity, and adherence. Its importance as a biological skin substitute when treating burns comes down to: control of water loss, a barrier to bacterial colonization, and acceleration of epithelialization.

The basic qualities which prove the usefulness of the amnion membrane as a biological skin substitute when treating burns are [11]: amnion membrane is not an immunogenic tissue; its bacteriostatic characteristic; its antiphlogistic characteristic; it reduces angiogenesis; it inhibits the fybroblastal function by reducing the cicatriciel tissue; it prevents the degradation of collagen; and it facilitates epithelialization.

Indications for the use of amnion membrane when treating burns are [12]: dermal burns (burns on the facial area, small burns, extensive burns); subdermal burns (before necrectomy, after necrectomy, over autografts (reticulated or microskin grafts)). In practice, two types of amnion membrane are used: *in toto* (amnion and chorion); and amnion only. The amnion membrane is an immunologically inert tissue, while the chorion causes an intense "host versus

graft" reaction [7]. The amnion membrane can be applied fresh or preserved. There are three basic ways to preserve the amnion membrane: in 76% alcohol; in 85% glycerol; and by irradiation with  $Co^{60}$ . The most frequently used is the method of preservation with 76% alcohol.

The aim of this study was to investigate the application of amnion membrane in the treatment of burns.

## Materials and Method

We conducted a prospective, randomized clinical trial over a period of two years at the Burns Centre, Department of Plastic and Reconstructive Surgery, at the St. Naum Ohridski Hospital for Surgical Diseases, Skopje. In this comparative study altogether 60 patients with dermal and subdermal burns were involved. Examinations were made of an *Examinational group* of 30 patients where the method of amnioplasty was applied and a *Control group* of 30 patients who were treated in a conventional way, by applying all standard methods for local treatment of burns (exposition, occlusive dressing and initial expansion with skin transplantation). Patients with fresh dermal and subdermal burns were included in the study. The basic criteria of selections were: gender, age, etiological factor, degree of burns and total body surface area (TBSA). Patients with burns admitted later than 24 hours after thermal trauma were excluded from the examination. Before the patients were involved in the study, written or oral agreements to be involved in the study were received from the patients.

Preserved amnion membrane in 76% alcohol was used for the amnioplasty. The placenta is first rinsed of blood with physiological solvate. Then the amnion membrane is separated from the rest of the chorion and is rinsed again. It is then preserved in 76% of alcohol, in sterile glass vessels. For the first 24 hours it is kept at room temperature, and then at +4°C. The expiry date is not limited. Before use, the vessel with the amnion membrane is placed in room temperature for ten minutes, and is then rinsed with sterile physiological solvate. The amnion membrane can be used for three months after it has been preserved. According to Shimazaki and his co-workers, the epithelium of the amnion membrane can manage to remain the same even 70 days after its presservation. After that period the epithelium cells become vacuolized, but remain attached to the basal membrane and the mesenchim. This method of preserving is the most acceptable, in terms of the technical preparation and the financial aspect. On the other hand, maximum security concerning the transmission of HIV virus, which is resistant of all types of rays but is not resistant to alcohol, is achieved. The temperature of +4°C in which the amnion membranes are kept causes a slowing down and quenching of the metabolic processes and reproduc-

tion of a large number of bacterial species, for example *Treponema pallidum* is extinguished after 2-3 days at  $+4^{\circ}$ C.

Preserved amnion membrane was applied over fresh dermal and subdermal burns (not later than 24 hours after the thermal trauma). In the case of the patients with dermal burns the change of biological skin substitutes was not necessary, while for the patients with subdermal burns there was a need for the frequent replacement of dried amnion membrane.

The dermal burns (Fig. 1) under the amnion membrane spontaneously epithelize, while with the subdermal burns the transplantation of the amnion was of a temporary character, until the moment when the condition of the patient was satisfactory for proceeding with skin autotransplantation [12].





Figure 1 – Extensive dermal burns treated with amnion membrane Слика 1 – Екстензивна дермална изгореница третирана со амнионска мембрана

In the case of burns of smaller extent, (Fig. 2) there was also a spontaneous epithelialization from the periphery to the centre, and the same was accelerated by the biological effect of the amnion membrane [13].

The clinical examinations were made on patients hospitalized in the Burn Centre at the St. Naum Ohridski Hospital for Surgical Deseases, Skopje. Pathohistological examinations on bioptical material were made at the Pathology Institute of the Medical Faculty in Skopje. Microbiological examinations of bioptical material were made at the Microbiology and Parasitology Institute of the Medical Faculty in Skopje.



Figure 2 – Patient with subdermal burns treated with amnion membrane Слика 2 – Пациент со субдермални и гореници третирани со амнионска мембрана

Pathohistological and microbiological analyses were made on bioptical material from the burned surfaces. The bioptical material was taken on the third day, and in the case of several examined patients on the seventh day after thermal trauma. Two bioptical full skin thickness segments  $4 \times 2$  mm were taken using a scalpel [14]. The degree of the burn was determined by pathohistological analysis of the bioptical material taken on the third day. In one group of the volunteers the re-epithelialization was determined on the seventh day (Fig. 3), and the further the re-epithelialization was clinically observed. Pathohistological examination enables discrimination between bacterial colonization and invasive bacterial infection.

The type of bacterial colonization or infection can be determined in the same way, which is also confirmed with microbiological analysis. The bioptical material is fixated for 24 hours in 10% of neutral puffered formalin. The excision of the skin is taken complete for routine processing, then it is moulded in paraffin blocks, then cut serially in findings which are 5  $\mu$ , and schiffed with haematoksilin-eozin. For visualization of the bacterial invasion Brown's and Brenn's histochemical schiffing was used. In several cases, histochemical PAS (periodic acid schiff) and trichrome schiff was used to prove the changes in the subepithelial connective tissue and the changes of the blood vessels.

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Figure 3 – Pathohistological preparation: completely finished re-epithelialization with adherent amnion membrane. Слика 3 – Патохис толошки препарат: целосно завршена реепителизација со адхерен тна амнионска мембрана

With the histological analyses the following changes are determined: the degree of the burn; the degenerative changes in the dermis; the presence of vital structures, skin adnexa; the degree of re-epithelialization after the application of amnion membrane; the vitality/disintegration of the amnion membrane; the presence of Gram-positive and Gram-negative bacteria/colonies of bacteria, and the depth of bacterial invasion: on the surface, epidermis, papillary/reticular subepidermis, subcutis fat tissue.

## Results

The total number of 60 analysed patients, 30 patients from the examination group who were treated with amnion membrane as a biological skin substitute and 30 patients from the control group who were treated with conventional methods for treating burns, was enough to give answers to the goals designnated for this clinical study.

Comparison of the patients from the control group with those from the examination group was tested using Student T-test for independent samples, or  $X^2$  for nominal parameters. The statistically important value of p was taken if p < 0.05.

Comparison of the age of the two groups with Student T-test for independent samples showed that there was no statistically significant difference, in

The advantages of the application...

other words we are talking about groups which are of approximately the same age. Revision of the significance was made by analyses of cross tables of a given parameter for both the groups (examination and control). We should emphasize that the cross tables were used when the parameters were given by category or descriptively.

For revision of the significance,  $X^2$  test was used, during which the hypothesis was verified that a given parameter did not have significant importance for the division of the number of patients in the two groups. Namely if, for example, gender does not have a significant influence, then it should be expected that the number of the patients according to gender is equally disposed in the two groups. There was no statistically significant difference according to the gender in the two groups, p = 0.598.

Both male and female patients were represented homogeneously in the two groups. A statistically important difference between the age groups, using the  $X^2$  test, was not obtained, meaning that according to age the patients were equally represented in the both groups p = 0.663.

The analyses of the etiological factor for the burns in the two groups showed no statistically important difference (Table 1).

Table 1 – Табела 1

Groups of patients according to the etiological factor Групи пациенти според е тиолошкиот фактор

Parameter	Category	Group of ve	Total	$\mathbf{X}^2$	
		Examination	Control		p value
	Hot water	11	9	20	
	Hot fluids	3	5	8	
Etiological	Flame	9	5	14	
Factor	Electricity	5	6	11	0.424
	Chemical	2	2	4	
	Other	0	3	3	
	Total	30	30	60	

The extent of the burned surface was compared with the  $X^2$  test in the two groups which were not different statistically, the groups are homogenous (Table 2).

The degree of the burn was determined with pathohistological analyses of the bioptical material taken from the burns the third day after the thermal trauma. The results showed that the number of patients is equally represented in the two groups according to the degree of the burns (Table 3).

Table 2 – Табела 2

Parameter	Category	Group of vo	olunteers	Total	$\mathbf{X}^2$
		Examination	Control		p value
	< 10%	7	13	20	
Extent of	10-20%	12	10	22	
burned	20-30%	5	3	8	
surface	30-40%	3	2	5	0.698
	40-50%	2	1	3	
	> 50%	1	1	2	
	Total	30	30	60	

Group of patients according to the extent of the burned surface Групи пациенти според степенот на изгорена површина

Table 3 – Табела 3

Group of patients according to degree of burns Групи пациенти с поред с тепенот на изгореници

Parameter	Category	Group of volu	nteers	Total	$\mathbf{X}^2$
		Examination Control			p value
Degree of burns	Dermal	9	10	19	
Pathohistological					
analyses	Sub dermal	21	20	41	0.781
Third day	Total	30	30	60	

The results of the microbiological and pathohistological examinations of the bioptical material taken on the seventh day and analysed in terms of bacterial colonization and infection showed that although there was no significant difference between the two groups (examinational and control), the value of p = 0.067 was very close to the statistically important value of p < 0.05 (Table 4). Because of the number of sterile findings, it can be concluded that for larger groups of patients there would have been significance.

The results of the pathohistological examinations of the bioptical material taken the seventh day and analysed according to the re-epithelialization, showed that there is significant difference between the two groups (examinational and control) of p < 0.035 (Table 5).

The application of the method of amnioplasty is approved, with the acceleration of the process of re-epithelialization in the treatment of burns.

## Table 4 – Табела 4

Tabular sum mary of microbiological and pathohistological analysis of bioptical material on seventh day in the two groups Табеларен Преглед на микробиолошка та и па тохис толошка та анализа на биоптички ма теријал на седмио т ден кај две те групи

Parameter	Category	Group of volunteers		Total	$\mathbf{X}^2$
		Examination	Control		p value
Microbiological					
and	Bacterial	9	16	25	
Pathohistological					
analyses	Sterile	21	14	35	0.067
Seventh day	Total	30	30	60	

Table 5 – Табела 5

Tabular summary of pathohistological analyses of the bioptical material according to re-epithelialization on seventh day in both groups.

Табеларен преглед на патохис толошки те анализи на биоптички материјал според реепитализација та на седмиот ден кај две те групи

Parameter	Category	Group of volunteers		Total	$\mathbf{X}^2$
		Examinational	Control		p value
	Absent				
Pathohistological	re-epithelialization	8	16	24	
	Present				
Analyses on	re-epithelialization	22	14	36	0.035
Seventh day	Total	30	30	60	

## Discussion

60 patients with burns were involved in the study. We should mention that the two groups analysed were identical (homogenous) in the number of patients, gender, age, etiological factor, extent of the burned surface and degree of burns, according to the  $X^2$  test, in other words we are talking about two identical groups.

The analyses of the results of the pathohistological examinations of the bioptical material taken and analysed the third day of the burn, and analysed according to the degree of the burn, showed that 21 (70%) of the burns were sub-

dermal, and 9 (30%) the burns were of dermal type. In the Control Group, 20 (66.66%) of the burns were subdermal, and 10 (33.33%) were of the dermal type.

Histological examination of the bioptical material taken from the burned surface is the only relevant parameter for determining the degree of the burn [15].

The analyses of the results from the microbiological and pathohistological examinations of the bioptical material taken on the seventh day and analysed for bacterial colonization and infection showed the presence of 21 (70%) sterile findings in the examination group and 14 (46.66%) sterile findings in the control group.

The statistical analyses of the microbiological and pathohistological examinations of the bioptical material taken the seventh day showed that althoughugh between the two groups (examination and control) there was no statistically important difference, the value of p = 0.067 is very close to the statistically important value of p < 0.05. However, we should mention that clinical significance was achieved.

The clinical use of the amnion membrane compared to the conventionally used methods in the treatment of the burns showed significantly better results, especially as regards bacterial colonization.

Clinically, in the examination group there were only signs of inflamematory reaction of the burned tissue, so only swelling, hyperaemia and haemorrhage could be seen. During the pathohistological analyses swelling, hyperaemia, haemorrhage and cellular infiltration, were morphologically present, which is in accordance with the data from the literature [16].

Tyszkiewicz in 1999 [17] published that, at the beginning of 1998, over 400 amnion allografts preserved with radiation sterilization, with a total surface of over 40,000 cm<sup>2</sup>, were prepared in the Central Tissue Bank in Warsaw. This method is superior compared to the conventional methods regarding bacterial colonization and the re-epithelialization of the burns. Ramakirshnan announced in 1997 that the use of amnion membrane preserved in 76% alcohol was an ideal method for preventing bacterial colonization in 350 cases [18].

In our study, in the patients treated with amnion membrane secretion was not present, especially not purulent secretion. This is because of the qualities of the amnion membrane. The amnion membrane is thin, transparent, semipermeabile, has the quality of adhering hard to the burned surface, and dries very fast. The dried amnion membrane decreases the possibility of bacterial colonization, and for already colonized bacteria it is not an appropriate medium for the growth and development of bacteria.

With the use of the amnion membrane as a biological skin substitute, the burned site is "closed" to bacterial colonization and "vertical" penetration of

bacteria into the tissue, and the development of exogenous bacterial infection is prevented.

The analysis of the results from the pathohistological examinations of the bioptical material taken on the seventh day after the skin was burned, and analysed with reference to re-epithelialization, showed that in the examination group re-epithelialization was present in 22 (73.33%) of the burns, and was absent in 8 (26.66%) patients. In the control group re-epithelialization started in 14 (46.66%), and was absent in 16 (53.33%) of the burns.

The statistical analysis showed that there was a significant difference between the pathohistological analysis of the bioptical material taken on the seventh day from both the groups (examination and control) with reference to the re-epithelialization of the burns of p < 0.035. The use of the method of amnioplasty is approved, because of the acceleration of the process of re-epithelialization of the burns.

### Conclusion

The histological analyses of the bioptical material not only determines the degree of the burns very precisely, but facilitates the choice of method for further treatment; it is possible to follow the speed of the re-epithelialization and this has a very important part in the correct diagnosis and the early starting of the specific therapy, important for the prevention of sepsis.

Applied amnion membrane as a biological skin substitute expedites reepithelialization and prevents invasive bacterial infection.

Pathohistological examination of burns is recommended to be introduced as a standard method in clinical practice.

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### Резиме

## ПРЕДНОСТ НА ПРИМЕНА НА АМНИОНСКАТА МЕМБРАНА ВО ТРЕТМАНОТ НА ИЗГОРЕНИЦИТЕ

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Суштински важен фактор за успешно лекување на изгорениците е раното препокривање на изгорената површина со кожни заместители. Препокривањето на изгорениците бара материјал кој ја реставрира епидермалната функција и се интегрира во процесот на заздравување. Биолошките преврски претставуваат златен стандард за времено препокривање на изгорениците. Сите биолошки кожни заместители се подложни на рана графт реакција, исклучок претставува амнионската мембрана. Значењето на амнионската мембрана како биолошка преврска при изгореници се сведува на: бариера за бактериска колонизација, забрзување на епителизацијата и контрола на водената загуба. Амниопластиката е метод на апликација на амнионска мембрана на "примачка регија" (recipient site).

Во оваа компаративна студија беа вклучени вкупно 60 пациенти со дермални и субдермални изгореници. Иследувањата беа изведени на испитувана група која ја сочинуваа 30 пациенти со изгореници кај кои се примени методата на амниопластика и при тоа беше користена амнионска мембрана конзервирана со 76% алкохол. Контролната група ја сочинуваа 30 пациенти со изгореници третирани конвенционално, така што беа применети сите стандардни методи за локално згрижување на изгорениците: експозиција, оклузивна преврска и иницијална екцизија со кожна трансплантација. Вршени беа патохистолошки и микробиолошки анализи на биоптичен мате-

ријал од изгорениците. Со патохистолошка анализа на биоптичниот материјал земен третиот ден се одредуваше степенот на изгорениците, кај одреден број испитаници седмиот ден се одредуваше реепителизацијата; понатаму реепителизацијата се следеше со клиничка опсервација. Патохистолошката егзаминација овозможи дискриминација помеѓу бактериската колонизација и инвазивната бактериска инфекција. Исто така се одредуваше и типот на бактериска колонизација или инфекција што се потврдуваше и со микробиолошка анализа.

Анализата на резултатите од микробиолошките и патохистолошките иследувања на биоптичниот материјал во однос на бактериска колонизација и инфекција покажа дека иако меѓу испитуваната и контролната група нема статистички значајна разлика, вредноста на р = 0.066807 е многу блиска до статистички значајната вредност на р < 0.05. Резултатите од патохистолошките иследувања на биоптичниот материјал земен седмиот ден и анализиран во однос на реепителизацијата, покажа дека постои сигнификантна разлика кај двете групи од р < 0.035023.

Треба да се напомене дека иако во однос на микробиолошките иследувања на биоптичниот материјал не е добиена статистички значајна разлика меѓу двете групи, постигната е клиничка сигнификантност. Добиената пак сигнификантност од р < 0.035023 во однос на реепителизацијата кај двете групи ја оправдува примената на метадата на амниопластика.

Хистолошката анализа на биоптичен материјал не само што го одредува прецизно степенот на изгорениците туку го олеснува изборот на метадата за понатамошен третман, се следи брзината на реепителизацијата и има значајна улога во точната дијагноза и раниот старт на специфичната терапија, значајна за превенирање на сепса. Применетата амнионска мембрана како билошка преврска ја забрзува реепителизацијата и ја спречува инвазивната бактериска инфекција. Патохистолошката егзаминација на изгорениците се препорачува да се воведе како стандардна метода во клиничката практика.

**Клучи зборови:** амнионска мембрана, изгореници, биопсија, атохистолошка егзаминација.

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