Research Article

https://doi.org/10.23934/2223-9022-2022-11-1-75-85

Skin Micrograph Protector in a Burn Wound: Alloderm or Hydrogel Coating?

I.Yu. Arefyev, M.G. Ryabkov✉, V.V. Beschastnov, M.L. Bugrova, K.S. Petrova, P.V. Peretyagin, L.B. Timofeeva, A.A. Karpenko, A.A. Tulupov, N.A. Koloshein

University Hospital
Privolzhsky Research Medical University
10/1 Minin and Pozharsky Sq., Nizhny Novgorod, 603005, Russian Federation

✉ **Contacts:** Maxim G. Ryabkov, Doctor of Medical Sciences, Senior Lecturer, Leading Researcher of the University Hospital, Privolzhsky Research Medical University.
Email: maxim-ryabkov@yandex.ru

AIM OF STUDY Was to compare the dynamics of engraftment of skin micrographs in a burn wound when using protectors from an allodermal graft and from a hydrogel coating.

MATERIAL AND METHODS The experimental study was conducted on 18 rats with a scab formed 3 days after modeling a deep burn with an area of 20% of the body surface. Partial fascial necrectomy was performed: two rounded sections of the sling with a diameter of 25 mm were excised. 6 automicrographs of skin 4x4 mm, 0.3 mm thick, were applied to each surface freed from the scab. In each animal, micrographs on one of the wounds were covered with a hydrogel protector, on the other with an allodermotransplant from another animal of the group. A secondary aseptic dressing was applied to the protectors. On the 5th and 20th days after the operation, the state of micrographs was studied: blood circulation — according to laser Doppler flowmetry, microstructure in vivo — using optical coherence tomography, microstructure ex vivo — according to histological examination of biopsies.

RESULTS Differences in the rate of restoration of blood circulation of micrographs in the early stages of the postoperative period were found. In the first 5 days, the perfusion of micrographs under an allodermal protector exceeded the indicator in micrographs under a hydrogel coating by 44 [21; 51] % (p=0.031) due to the contribution of endothelial and neurogenic mechanisms of blood flow modulation. Starting from day 10, the differences in perfusion were levelled, but there were signs of more active endothelial regulation of blood flow under the skin (p=0.028). Histologically, the appearance of full-blooded capillaries was revealed earlier in micrographs under the alloderm than when using a hydrogel protector. By 20 days, under the condition of regular change of hydrogel coatings, the area of wound healing under the studied coatings did not significantly differ. However, the structure of the integumentary tissue under the alloderm according to the optical coherence tomography data was closer to normal skin than when using a hydrogel protector.

CONCLUSIONS

From the point of view of the physiology of the wound process, alloderm is the preferred option of an autograft protector in comparison with a hydrogel coating, which is probably due to the paracrine biological activity of the alloderm. However, hydrogel coatings can provide a comparable level of efficiency, provided they are regularly changed and, potentially, given the properties of cytokine activity.

Keywords: burn, shortage donor site split skin, alloskin graft, hydrogel dressing, skin micro-grafting, autoskin grafting, laser doppler flowmetry, optical coherence tomography, wound dressing

For citation Arefyev IYu, Ryabkov MG, Beschastnov VV, Bugrova ML, Petrova KS, Peretyagin PV, et al. Skin Micrograph Protector in a Burn Wound: Alloderm or Hydrogel Coating? *Russian Sklifosovsky Journal of Emergency Medical Care*. 2022;11(1):75–85. DOI: 10.23934/2223-9022-2022-11-1-75-85 (In Russian)

Conflict of interest Authors declare lack of the conflicts of interests

Acknowledgments, sponsorship Ministry of Health of the Russian Federation. EGISU registration number: 121022500010-6

Affiliations

Igor Yu. Arefyev	Candidate of Medical Sciences, Director of the University Hospital, Head of the Burn Center, Privolzhsky Research Medical University; https://orcid.org/0000-0003-1959-2766, igor_arefev@pimunn.ru; 20%, concept development, final approval of the manuscript
Maksim G. Ryabkov	Doctor of Medical Sciences, Associate Professor, Leading Researcher, University Hospital, Privolzhsky Research Medical University; https://orcid.org/0000-0002-9555-190X, maxim-ryabkov@yandex.ru; 20%, concept development and study design, analysis and interpretation of all data, drafting of the manuscript
Vladimir V. Beschastnov	Doctor of Medical Sciences, Associate Professor, Leading Researcher, University Hospital, Privolzhsky Research Medical University; https://orcid.org/0000-0002-9332-3858, vvb748@mail.ru; 11%, interpretation of the received data, drafting of the manuscript
Marina L. Bugrova	Doctor of Biological Sciences, Associate Professor, Head of the Department of Histology, Cytology and Embryology, Privolzhsky Research Medical University; https://orcid.org/0000-0002-8294-4595, marysmir@mail.ru; 9%, material analysis and interpretation of histological data

Kseniya S. Petrova	Candidate of Medical Sciences, Associate Professor, Department of Skin and Venereal Diseases, Privolzhsky Research Medical University; https://orcid.org/0000-0002-4024-470X, doctorks@yandex.ru; 9%, conducting physical research methods, material analysis and interpretation of OCT data
Peter V. Peretyagin	Junior Researcher, Department of Physical and Chemical Research, Central Scientific Research Laboratory, Privolzhsky Research Medical University; https://orcid.org/0000-0003-0707-892X, peretyaginpv@gmail.com; 8%, conducting physical research methods, material analysis and interpretation of LDF data
Lydia B. Timofeeva	Candidate of Biological Sciences, Senior Lecturer, Department of Histology, Cytology and Embryology, Privolzhsky Research Medical University; https://orcid.org/0000-0002-8191-7739, bioli@mail.ru; 8%, material analysis and interpretation of histological data
Artem A. Karpenko	Resident, Department of Skin and Venereal Diseases, Privolzhsky Research Medical University; https://orcid.org/0000-0002-5452-3021, shotokan0307@mail.ru; 6%, OCT data collection, OCT data processing
Alexander A. Tulupov	Junior Researcher, University Hospital, Privolzhsky Research Medical University; https://orcid.org/0000-0001-6567-7803, tulupov.a.a@yandex.ru; 5%, collection of experimental material, statistical processing
Nikita A. Koloshein	Junior Researcher, University Hospital, Privolzhsky Research Medical University; https://orcid.org/0000-0002-4563-5478, n.koloshein@mail.ru; 4%, collection of experimental material, database formation

LDF – laser doppler flowmetry
OCT – optical coherence tomography
MP – microcirculation parameter

INTRODUCTION

More than 300,000 cases of thermal and chemical burns are registered annually in Russia, and 45,000–47,000 patients with the most severe injuries require specialized inpatient treatment [1]. At the same time, in terms of the severity of injury, mortality and disability, as well as the financial costs of treatment and rehabilitation, thermal injuries rank first among other injuries [2, 3].

Autodermoplasty remains the gold standard for skin restoration in burn injuries. However, with a full thickness burn area of more than 25% of the body surface, there is a need for additional materials for temporary or permanent wound closure. To overcome the shortage of plastic material, among other things, the technique of skin automicrografting has been developed and used since 1958 [4, 5].

Despite the fact that the graft area can be expanded to a ratio of 1:6–1:9, skin micrografting is used much less frequently than traditional plastic surgery with a split-thickness skin graft [6]. One of the reasons is the need to maintain a constancy of the environment around the micrografts which are sensitive to desiccation and infection. A proven solution to this problem and a routine practice in the countries of the EU, the Middle East, and the Asia-Pacific region [7] is the use of alloskin as a temporary cover material for micrografts on the wound surface. The amount of alloskin used is calculated in tens of square meters per year in Italy [8], hundreds of square meters - in China [9], Japan [10], India [11], Iran [12], Poland [13].

It should be emphasized that split-thickness grafts from cadaveric, salvaged, “live” alloskin have been massively used in the USSR and the Russian Federation for decades [14, 15].

The properties of alloskin as a temporary cover for burn wounds are well studied, and technologies for its use are being developed [16, 17]. However, changes in the domestic regulatory framework have led to a rapid reduction in the use of alloskin in this country; its use in routine clinical practice is possible only in few large clinical centers [14].

Promising synthetic “substitutes” for allodermal protectors include modern hydrogel coatings with a three-dimensional mesh structure synthesized via natural or synthetic polymers [18, 19]. Hydrophilic coatings absorb wound exudate, maintain the humidity of the environment necessary for regeneration in the wound [20].

A comparative study of the dynamics of engraftment of skin micrografts under allodermal and hydrogel coatings was carried out for the first time. It is especially relevant for Russian combustiological science and practice, as it is designed to fulfill several important tasks: to increase knowledge about the properties of allo-skin obtained by modern bioimaging methods; to clarify ways to improve hydrogel wound dressings. The subject of our study was dynamic changes in microcirculation, the microstructure of skin micrografts in a burn wound under two different protectors - a split-thickness skin allograft or a hydrogel wound dressing.

The aim of the study was to compare the dynamics of engraftment of skin micrografts in a burn wound when using allodermal graft and hydrogel coating protectors.

MATERIAL AND METHODS

Our experimental study was carried out on outbred Wistar rat models weighing from 235 to 355 g (males, n=18). All the animals for the experiment were selected from different litters, they were not related. The study was approved by the Local Ethics Committee of the Federal State Budgetary Educational Institution of Higher Education "Privolzhsky Research Medical University" of the Ministry of Health of Russia, Protocol No. 13 dated 05.11.2015. The procedures were performed under general anesthesia with 3.5% tiletamine hydrochloride in combination with 2% xylazine hydrochloride which were administered to the animals intramuscularly. During the experiment, the animals were simulated contact full thickness burns of 20% of the body surface. 3 days after the injury, a partial fascial necrectomy was performed: two rounded sections of the sling with a diameter of 25 mm were excised. Immediately after the necrectomy, 6 automicrographs of skin (hereinafter referred to as micrografts) of 4 × 4 mm, 0.3 mm thick, were applied to the wound surface. In each animal, micrografts on one wound were closed with a split-thickness allodermograft taken from another animal from the group (AlloS wounds), and on the other wound, with a hydrogel wound dressing (H/Gel wounds) (Fig. 1). The polyvinyl alcohol hydrogel coating had a thickness of 45–65 μm, a degree of hydrolysis of 99.4 ± 0.4%, and pH of 6.6–7.8. An aseptic bandage was applied over the allodermal or hydrogel protector.

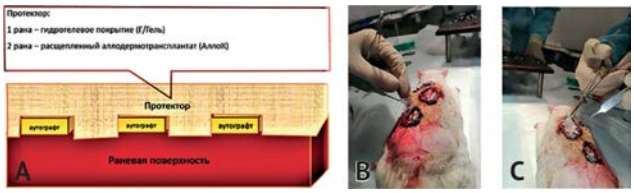

Fig. 1. Stages of the experiment, autodermal graft transplantation and protectors on the wound surface

A – scheme of the combined use of autodermal grafts and protectors; B – autodermal graft closure with a hydrogel wound dressing; H/Gel wounds; C –autodermal graft closure with skin allograft; AlloS wounds

On the 5th and 20th days of the postoperative period, the condition of skin micrografts was studied: blood circulation – according to laser Doppler flowmetry, in vivo microstructure – using optical coherence tomography, ex vivo microstructure - according to histological examination of biopsy specimens (Fig. 2).

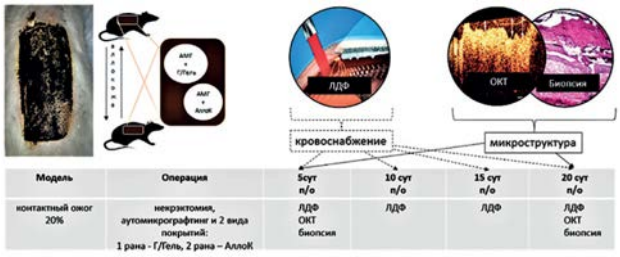

Fig. 2. Experiment scheme

Notes: ЛДФ — laser Doppler flowmetry; ОКТ — optical coherence tomography

To do this, on the 5th day of the postoperative period, all the animals underwent in vivo laser Doppler flowmetry (LDF) and optical coherence tomography (OCT) of the micrografts and underlying tissues, and then peri-wound tissues with micrografts were excised in 50% (9 out of 18) of the animals and these animals were taken out of the experiment.

On the 20th day of the postoperative period, the remaining 9 rats underwent LDF, OCT, and excision of micrografts for histological examination, after that they were taken out of the experiment.

METHODS FOR STUDYING THE WOUND HEALING PROCESS

Laser Doppler flowmetry. To assess the microcirculation in the zone of micrografts, laser Doppler flowmetry was used (Lasma-MC device, manufacturer: Scientific Productive Enterprise LAZMA, Russia). The depth of penetration of the probing radiation into the tissues was 1 mm (0.35 mm is the thickness of the micrograft + 0.65 mm of the underlying tissues). The parameters of basal blood flow were recorded (microcirculation indicator — MI; the variable component of the signal — the root-mean-square deviation of perfusion fluctuations δMI). To assess the mechanism of micrograft reperfusion, we compared the contribution of active microcirculation control factors in the frequency range of 0.005–0.12 Hz (endothelial, neurogenic and myogenic factors of the intrinsic activity of the vascular wall) and passive control factors in the range of 0.2–1.6 Hz (external components of blood flow regulation (respiratory and cardiac ones).

Optical coherence tomography. For structural and anatomical analysis of the micrografts, we used a spectral multimodal OCT device (Federal Research Center "Institute of Applied Physics, Russian Academy of Sciences", Nizhny Novgorod, Russia) with a wavelength of probing polarized radiation of 1300 nm. The longitudinal resolution of the system is 10 μm, the depth resolution is 15 μm, the scanning depth is 1.5 mm. Scanning speed 20,000 A-scans/sec. The size of the images obtained within 26 seconds is 2.4x2.4x1.5 mm.

Histological examination. A biopsy of the micrograft with adjacent wound tissues and coating was performed in 9 animals on the 5th and in the remaining 9 animals on the 20th day of the experiment. The tissue was fixed in 10% phosphate-buffered (pH 7.2) formalin, then subjected to standard histological processing and embedded in HISTOMIX-extra paraffin-based embedding medium (Biovitrum, Russia) or TissuePrep 2 (Fisher Scientific, USA). On a Leica SM 2000 R microtome, histological sections of the central region of the wounds were made in the transverse plane with a thickness of 5 μm. For morphological examination, sections were stained with hematoxylin-eosin. The preparations were studied using a Nikon Eclipse 80i microscope, photographic recording was performed with a Nikon Ds-Fi1 camera, and panoramic images were done using the Nis-Elements BR imaging software.

STATISTICAL DATA PROCESSING

Statistical processing of the obtained data was performed using the STATISTICA 10 software package (StatSoft, Inc., USA) and the Prism 6 program (GraphPad Software, USA). The assessment of the statistical significance of differences when comparing groups by quantitative characteristics was carried out using nonparametric methods. The Mann-Whitney test was used to compare the parameters in the groups. Confidence intervals for relative indicators were estimated using the Wilson method. The sample parameters given below have the following designations: Me — median, Q1 — upper quartile, Q3 — lower quartile, minimum (min) and maximum (max) — minimum and maximum values of the variable, n — size of the analyzed subgroup, p —value of the statistically significant difference. The critical significance level was taken equal to 5% ($p \leqslant 0.05$).

RESULTS

Dynamics of engraftment of autodermal grafts: data from visual observation and structural OCT analysis of tissues obtained in vivo. In the first 5 days of the postoperative period, the area of the wounds with both types of protectors under study did not decrease. Micrografts remained viable, but adhesion to the wound surface was weak. In the AlloS wounds, during the first 5-10 days of observation, the allodermal protector retained its elasticity, physiological color, and adhered tightly to the wound surface. By the 15th day of the postoperative period, all animals developed signs of its degradation: the AlloS protector lost moisture, elasticity, became brittle, acquired a predominantly brown-cyanotic color and marginal foci of lysis appeared. The peak of degradation manifestations occurred on the 12–15th day. All micrografts placed on the AlloS wounds by this time were viable, fixed to the wound surface. In 6 out of 9 animals, autodermal grafts were an overgrown “tile-like” layer of integumentary tissue on the wound (Fig. 3b, 4A).

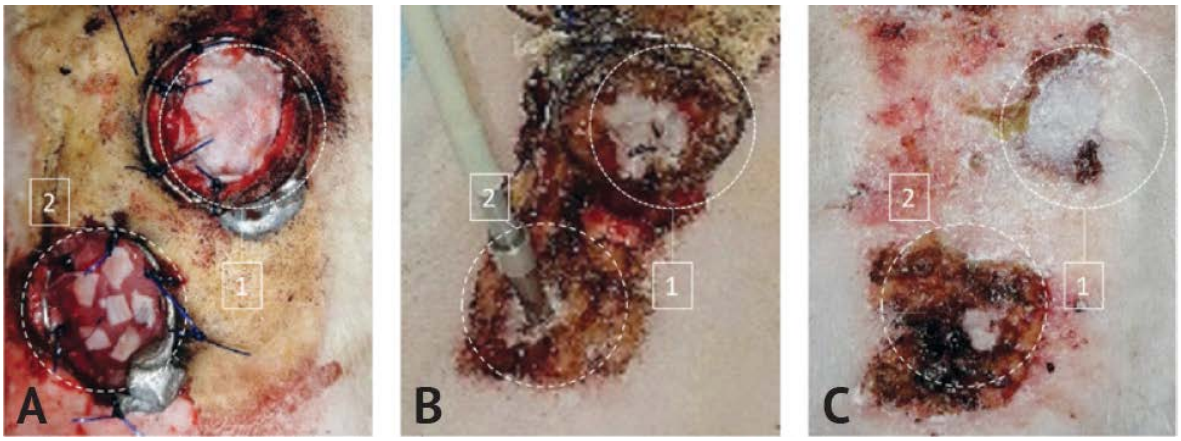

Fig. 3. Dynamics of autodermal engraftment in a burn wound with hydrogel (2) and allodermal (1) protectors. A — 1st day after surgery; B — 15th day after surgery; C — 20th day after surgery

By the 20th day of observation, on the surface of wounds under the AlloS allodermal protector viable micrografts surrounded by areas of marginal epithelization occupying a total of 87 [81; 95] % of the wound were visualized (Fig. 4 А, Б). As could be seen on the obtained during the experiment optical coherence tomograms of the wound surfaces with micrografts covered by the allodermal protectors, during 20 days of observation the space between the micrografts gradually decreased due to their engraftment, marginal epithelization, and contraction of the wound.

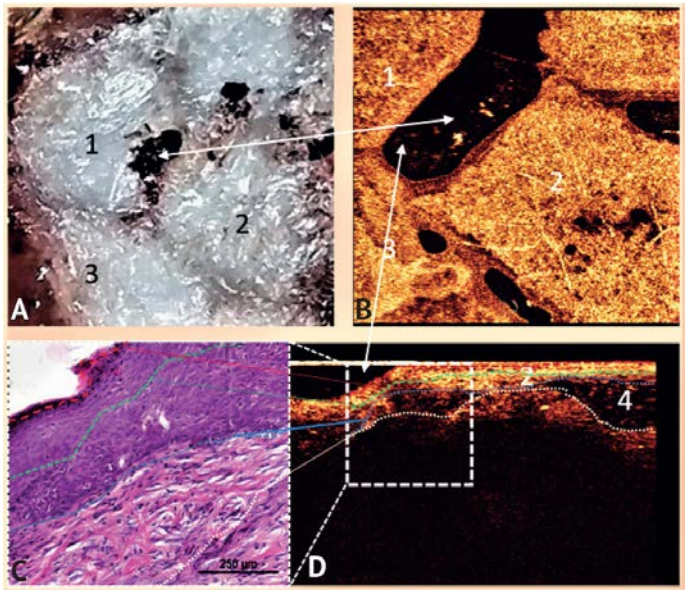

Fig. 4. Wound appearance (AlloS) on the 20th day of observation. A — photo of micrografts on the AlloS wound; B — en-face OCT imaging of micrografts on the AlloS wound; C — histological section of the wound surface, micrograft on the AlloS wound, ×100; D — OCT imaging of micrografts on the AlloS wound, sagittal view. 1, 2, 3 — micrografts, 4 — liquid micro accumulations in the contact zone of the micrografts and the recipient wound. The layers of the skin microstructure are indicated by the dotted lines: red — the stratum corneum of the epidermis, green — the squamous cell layer of the epidermis, blue — the zone of the dermoepidermal junction, yellow — the zone of contact with the recipient surface

According to OCT data, the stratum corneum of the epidermis, the suprapapillary cellular layer of the epidermis, and the zone of the dermoepidermal junction were differentiated in the structure of micrografts and in the areas of secondary marginal epithelization (Fig. 4 В, Г). In the contact zone of the micrograft and the recipient wound, according to OCT data, optical equivalents of liquid micro accumulations were visualized — areas of low optical density ranging in size from 50 to 470 µm (Fig. 4 Г).

On the wounds covered with a hydrogel coating, the dynamics appeared to be similar: the micrografts remained viable, their adhesion to the wound surface was weak. At the same time, the hydrogel protectors on

the wounds lost moisture and transparency, became brittle and fragmented already by the 5th day of observation. Starting from the 5th day of the postoperative period, hydrogel coatings were changed every 5 days to maintain a moist environment in the wound.

By the 20th day after the operation in the H/gel wounds, the skin micrografts externally represented a continuous layer of integumentary tissue with focal defects, the total area of the closed wound surface was 81 [75; 92] % and did not differ from this indicator on AlloS wounds (p=0.781). At the same time, visually and OCT-determined zones of contact between micrografts were preserved (Fig. 5).

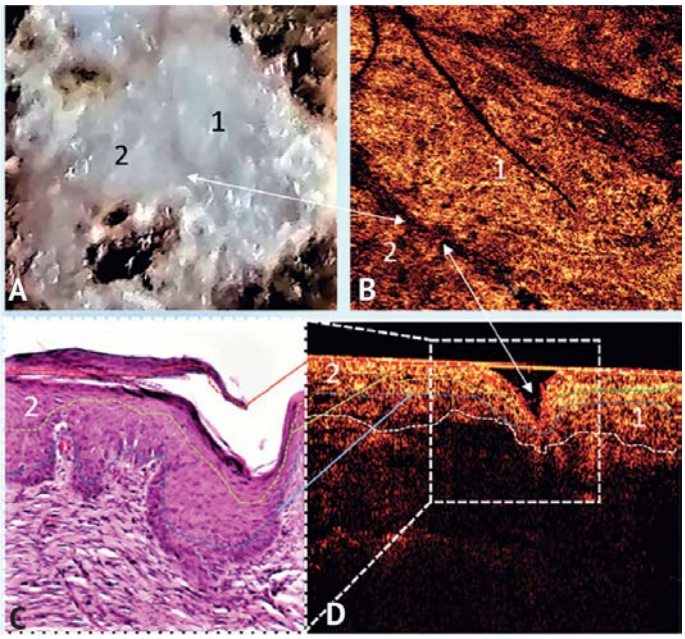

Fig. 5. Wound appearance (H/Gel) on the 20th day of observation. A — photo of micrografts on the H/Gel wound; B — en-face OCT imaging of micrografts on the H/Gel wound; C — histological section of the wound surface, micrograft on the H/Gel wound, ×100; D — OCT imaging of micrografts on the H/Gel wound, sagittal view. 1, 2 — micrografts, the layers of the skin microstructure are indicated by the dotted lines: red — the stratum corneum of the epidermis, green — the squamous cell layer of the epidermis, blue — the zone of the dermoepidermal junction, yellow — the zone of contact with the recipient surface

The full-thickness microstructure of the skin according to OCT data was determined only in the central zones of the micrografts. In transitional zones with marginal epithelialization, despite the visually indistinguishable picture, a full- thickness skin microstructure with five clearly differentiated layers was not detected.

Microcirculation according to LDF. In both groups of wounds, the blood flow parameters progressively changed during the entire observation period (Table 1). At the same time, the rate of blood circulation restoration and the mechanisms of blood flow regulation at different stages of the post-transplantation period differed and depended on the wound protector type. Statistically significant differences between blood circulation in skin micrografts covered with the hydrogel dressing and alloskin, judging by the integral microcirculation parameter (MP), were detected on the 5th day of the postoperative period. In the H/Gel group wounds, the median MP was 12.7 [11.2; 14.7] perfusion units (PU), and in the wounds of the AlloS group - 18.3 [16.5; 19.5] PU. (p=0.031). Judging by the LDF data, the growth of MP in the AlloS wounds as compared to the H/Gel wounds occurred due to a more pronounced blood flow modulation, the parameter σ during this period of observation in wounds under alloskin was 5.2 [4.5; 5.6] PU, and under the hydrogel coating - 2.4 [2.4; 3.1] PU (p=0.002).

Table 1

Microcirculation parameters (Me [Q1; Q3]) in the micrografts and the tissue surrounding the burn wound under the hydrogel protector ("H/Gel") and under the allodermal protector ("AlloS")

Laser Doppler flowmetry data	Wound	Follow-up period after transplantation, days			
		5	10	15	20
Microcirculation parameter MP (PU)	H/Gel	**12.7 [11.2; 14.7]**	14.7 [12.1; 15.1]	13.6 [11.9; 15.6]	14.9 [13.1; 16.0]
	AlloS	**18.3 [16.5; 19.5]**	16.6 [13.2; 16.9]	13.1 [11.2; 14.4]	15.3 [13.8; 17.1]
	p	**0.031***	0.094	0.786	0.357
Standard deviation of perfusion changes, σ (PU)	H/Gel	**2.4 [2.4; 3.1]**	3.3 [3.2; 3.8]	4.8 [4.3;5.2]	5.6 [5.0; 5.8]
	AlloS	**5.2 [4.5; 5.6]**	4.6 [3.9; 4.9]	4.4 [4.1;4.8]	4.1 [3.9; 4.9]
	p	**0.002***	0.072	0.367	0.784
Endothelial modulation, E (0.007–0.017 Hz)	H/Gel	**4.0 [3.6; 4.6]**	**4.6 [4.2; 5.8]**	5.9 [4.1; 6.0]	5.8 [5.2; 6.7]
	AlloS	**4.9 [4.2; 5.4]**	**6.3 [5.2; 6.8]**	6.3 [5.7; 6.5]	6.9 [6.3; 8.2]
	*p	**0.044***	**0.028***	0.080	0.137
Neurogenic modulation, N (0.023–0.046 Hz)	H/Gel	**3.4 [3.3; 3.7]**	3.5 [3.3; 3.8]	2.2 [2.2; 3.8]	3.5 [2.7; 3.9]
	AlloS	**4.8 [4.5; 4.9]**	4.0 [3.8; 4.6]	2.7 [2.4; 3.1]	4.1 [3.9; 4.9]
	p	**0.014***	0.167	0.867	0.127
Myogenic modulation, M (0.07–0.12 Hz)	H/Gel	5.5 [5.1; 6.4]	5.5 [4.6; 7.0]	6.7 [5.7; 7.9]	8.1 [6.6; 9.1]
	AlloS	6.6 [6.1; 7.4]	5.9 [4.3; 7.7]	6.1 [5.0; 7.5]	7.5 [5.2; 8.2]
	p	0.225	0.772	0.480	0.127
Respiratory modulation, R (0.2–0.4 Hz)	H/Gel	11.4 [9.3; 14.5]	10.4 [8.4; 12.2]	8.1 [7.0; 9.0]	9.3 [8.6; 9.9]
	AlloS	12.7 [10.5; 14.9]	9.9 [7.7; 12.7]	10.1 [7.8;12.4]	8.7 [8.0; 9.8]
	p	0.337	0.867	0.078	0.634
Cardiac modulation, C (0.8–1.6Hz)	H/Gel	7.2 [6.2; 8.1]	5.8 [4.5; 7.0]	6.4 [5.1; 7.2]	5.1 [4.2; 6.0]
	AlloS	8.1 [7.1; 9.9]	7.1 [4.9; 8.0]	6.2 [5.5; 7.5]	5.8 [4.8; 7.0]
	p	0.132	0.087	0.995	0.235

Notes: * — the differences between the parameter values in the "H/Gel" and "AlloS" wounds according to the Mann-Whitney test are statistically significant

In the mechanism of temporal variability of blood flow in the AlloS wounds, a significantly greater contribution than in the H/Gel wounds was made by active modulation factors: during the first 5 days - endothelial and neurogenic (p = 0.044 and 0.014, respectively), within 10 days — endothelial (p=0.028) (Table 1).

Starting from the 10th day of observation, the microcirculation parameter (MP) in micrografts under the studied protectors did not differ statistically significantly, the differences in the parameter of the standard deviation of perfusion changes were leveled off. However, for another 5 days, a pattern of higher activity of the endothelial factor of blood flow modulation persisted.

According to the histological examination ex vivo, the microstructure of the autodermal grafts, in general, testified to the successful engraftment of skin micrografts under both studied types of protectors by the 20th day of the postoperative period. However, the intermediate parameters of the histological picture obtained on the 5th day of the postoperative period differed (Table 2).

Table 2

Histological structure of the micrografts on the surface of a burn wound under hydrogel ("H/Gel") and allodermal ("AlloS") protectors on the 5th day of observation

Wound protector	Characterization of histological preparations		
	Protector state	The state of the underlying tissues	Micrograft state
H/Gel	Fragmented, covered with detritus (Fig. 6.1.2)	Blood filling of capillaries "+/-"; infiltration - mononuclear leukocytes; severe edema (Fig. 6.1.1, 6.1.3)	The structure is preserved, there are dystrophic changes in the cells, a meager amount of filled capillaries (Fig. 6.1.1., 6.1.2, 6.1.3)
AlloS	The integrity is preserved, the structure of the skin is mostly lost (Fig. 6.2.2)	Blood filling of capillaries "++"; infiltration - mononuclear, segmented leukocytes; severe edema (Fig. 6.12.1, 6.2.3)	The structure is preserved, mitotic figures in the basal layer, dystrophic changes in the cells, a moderate amount of filled capillaries (Fig. 6.2.1., 6.2.2, 6.2.3)

In the H/Gel wounds on the 5th day of observation in sections of micrografts and underlying tissues, the following was noted: the presence of newly formed connective tissue; moderate polymorphocellular infiltration; a small number of perfused blood vessels (Fig. 6a). In 3 out of 9 preparations, a layer of structureless tissue detritus between the hydrogel protector, micrograft and underlying tissues was determined. The hydrogel coating was fragmented and dehydrated. In the tissues of 2 preparations, foci of hemorrhage and areas of necrosis were found. In the structure of all micrografts, the epidermis and dermal layers were distinguished. However, in the cells of the spinous layer of the epidermis there were signs of dystrophic changes, hyperchromia and lysis of the nuclei, vacuolization and destruction of the cytoplasm were observed. Edema, single capillaries and small areas of hemorrhage were revealed in the papillary dermis. The hair follicles in the reticular layer were preserved. In the tissue adjacent to the micrograft, infiltration of polymorphonuclear leukocytes was observed, under the micrograft – mononuclear leukocytes (Fig. 6).

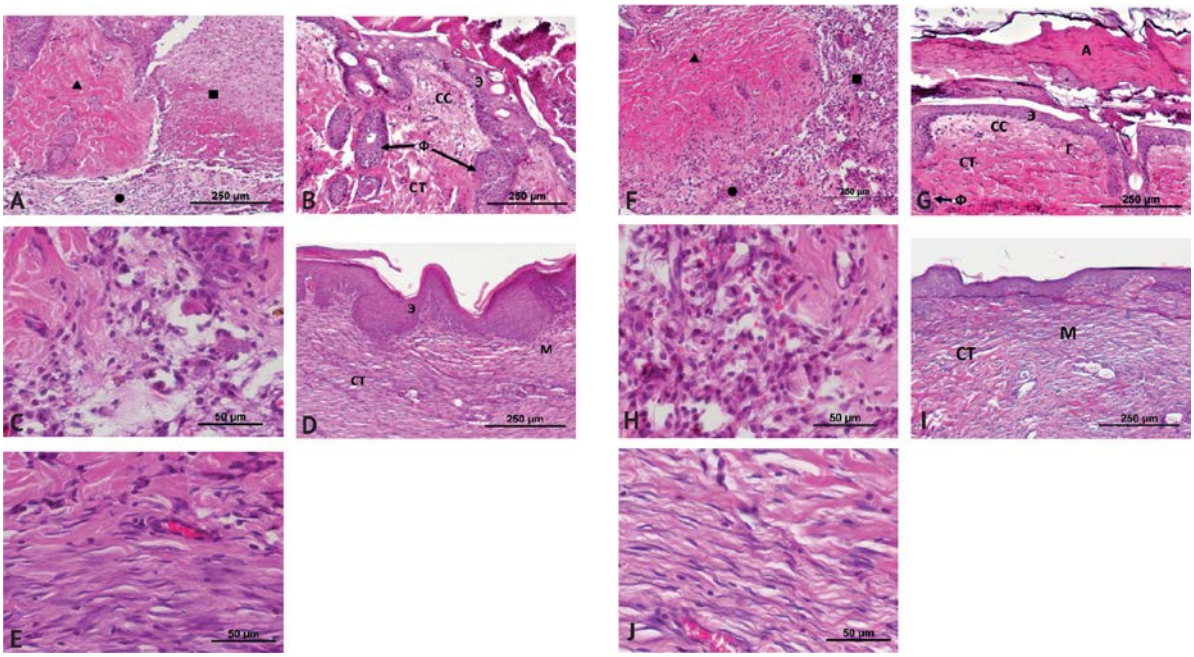

Fig. 6. Microstructure of the micrografts and tissues of the burn wounds. A — the micrograft and underlying tissues, H/Gel wound, 5th day, ×100; B — the micrograft under hydrogel protector, fragmented protector, detritus above micrograft, H/Gel wound, 5th day, ×100; C — mononuclear leukocytes under the micrograft, H/Gel wound, 5th day, ×400; D — the micrograft in the H/Gel wound, 20th day, ×100; E — tissue under the epithelium, H/Gel wound, 20th day, ×400: fibroblasts predominate, a small number of mononuclear leukocytes, segmented leukocytes are sporadic; F — the micrograft and underlying tissues, AlloS wound, 5th day, ×100; G — the micrograft under the allodermal protector, the structure of the alloderm is partially preserved, AlloS wound, 5th day, ×100; H — mononuclear leukocytes under the micrograft, segmented leukocytes are sporadic, perfused capillaries, AlloS wound, 5th day, ×400; I — the micrograft under allodermal protector, AlloS wound, 20th day, ×100; J — tissue under the epithelium, allo-skin coating, AlloS wound, 20th day, ×400: fibroblasts predominate, a small number of mononuclear leukocytes, segmented leukocytes are sporadic. Hematoxylin-eosin stain.

Notes: А — allodermal protector, Э — epidermis, СС — papillary layer; СТ — reticular dermis, Ф — hair follicle, Г — hemorrhage in the micrograft tissue; ▲ — autodermograft; ■ — tissue of the recipient surface of the wound; ● — the wound bed

In the wounds of the AlloS group on the 5th day of the postoperative period, in all preparations, the germination of the underlying tissue into the micrograft was revealed, which indicated its engraftment. Under all the micrografts, a layer of newly formed connective tissue with the developed intercellular matrix, a large number of fibroblasts and leukocyte infiltration was observed. At the same time, hemorrhagic phenomena were relatively more pronounced: in 6 out of 9 preparations, foci of hemorrhage on the wound surface and between the micrograft and the underlying tissue were determined (Fig. 6; 2.2).

The structure of the micrograft in the AlloS wounds on the 5th day of observation included clearly differentiated epidermis and dermal layers. In most cells of the spinous layer of the epidermis, hyperchromia or lysis of the nuclei was noted. Single mitotic figures were visualized in the cells of the basal layer. In the papillary layer of the dermis, full-blooded capillaries, focal edema and small areas of hemorrhage were visible. Compared to the H/Gel wounds, in the underlying tissues of the AlloS wounds, infiltration with agranulocytes was more pronounced, a moderate amount of filled capillaries was observed.

On the 20th day of the postoperative period, in the samples under alloskin protector, a layer of newly formed epidermis on the wound surface was also observed. The structure of the underlying connective tissue was similar to that of the dermis in H/Gel wounds (Fig. 6.2.4). In the newly formed epidermis, the basal, spinous and granular layers were determined. In the basal and spinous layers, the shape of the cells was changed compared to the norm: in some cells, nuclei with pyknosis were detected, in others, lysis of the nucleus and cytoplasm was noted. The dermo-epidermal junction, as in the wounds with the hydrogel protector, was also damaged.

DISCUSSION

The results obtained indicate that during the first 5–10 days of the postoperative period microcirculation is more actively restored in the micrografts under an allodermal protector than under a hydrogel wound dressing. These data confirm a recently expressed point of view about the significant role of the biological activity of the dermal graft placed on the wound. Growth factors that accelerate wound healing, such as epidermal growth factor (EGF), fibroblast growth factor (FGF), transforming growth factor beta (TGFβ), platelet-derived growth factor (PDGF), stromal cell-derived factor 1 (SDF-1) [21, 22] are secreted by keratinocytes, fibroblasts, endothelial cells of the skin, even if we plan to use the allograft only as a mechanical and thermal protector of the wound surface. A weak point in the targeted use of the paracrine activity of alloskin in the treatment of burn wounds is the fact of rapid degradation of cytokines under the action of proteolytic enzymes [21, 22], which is quite consistent with the experimental data: the contribution of active blood flow modulation factors to the restoration of blood circulation under the alloderm exceeded that in wounds under cytokine-inactive hydrogel coating only in the first 5 days.

Hydrogel coatings as protectors of micrografts generally showed functional viability, albeit subject to certain mandatory conditions. An important difference between a hydrogel dressing and an allodermal protector, demonstrated by our study, is that the hydrogel dressing has limited hydrophilic properties and requires periodic replacement [23].

In addition, the hydrogel inherent properties are insufficient to perform a full-fledged barrier function, especially in relation to hospital microflora, which forces developers to combine hydrogel dressings with antibiotics and bacteriophages [24, 25].

The presence of a wound protector, regardless of its composition, actualizes another complex practical problem, the solution of which was proposed in our study - the objectification of the control of micrografts and the protector in vivo. So far, there have been few attempts to use bioimaging tools to solve this problem. We were able to obtain data on the dynamics of the OCT picture of skin micrografts and compare them with the histological picture. On the whole, the algorithm of OCT control of the micrograft condition that we developed is based on 20 years of fundamental work of domestic researchers [26–28]. At the same time, it undoubtedly has an innovative character; on a more extensive material, it significantly develops the conclusions of the innovative work of our colleagues from the USA, published this year [29]. The conducted research shows that OCT has the potential to visualize and measure the characteristics of a split-thickness skin graft, which is fundamentally important for practical combustiology and can be recommended in further studies.

CONCLUSION

C From the point of view of the physiology of the wound healing process, alloskin is the preferred variant of the autograft protector in comparison with the hydrogel coating, which is largely due to the paracrine biological activity of the alloderm.

The advantage of the alloskin protector is the rapid restoration of the barrier function of the missing skin, in part because of the absence of the need for periodic dressing changes. At the same time, without a fundamental solution to the problem of rejection of allogeneic skin, the prospects for its use are very limited. However, hydrogel coatings can provide a comparable level of effectiveness if they are regularly changed and, potentially, imparted with the properties of cytokine activity, enhanced barrier function, and antimicrobial activity.

Regardless of the protector composition, a significant potential for the use of optical coherence tomography for visualization and measurement of the characteristics of a split-thickness skin graft has been shown, which can be recommended for practical combustiology and experimental studies in dermotransplantology.

FINDING

1. In the first 5 days after transplantation of skin micrografts, blood circulation in them was restored faster under the allodermal protector than under the hydrogel coating. These differences were confirmed by a 1.4 times higher perfusion index (p=0.031) and a special ratio of endothelial and neurogenic mechanisms of blood flow modulation. Starting from the 10th day, the differences in perfusion leveled off.

2. According to the histological examination, differences in the microstructure of micrografts under the alloderm and under the hydrogel coating remained up to 10 days of observation. An earlier appearance of full-blooded capillaries was revealed under the allodermal protector. By the 20th day, according to OCT data, despite the absence of significant differences in the area of wound healing, the structure of the integumentary tissue under the allodermal protector was closer to normal skin than under the hydrogel protector.

REFERENCES

1. Alekseev AA, Kozhemyakina VV, Malyutina NB, Bobrovnikov AE. Optimization of Outcomes of Skin Restoration in Patients with Deep Burns. *Lechenie i profilaktika*. 2020;10(1):73–79. (in Russ.)
2. Alekseev AA, Bobrovnikov AE, Malyutina NB. Emergency and Emergency Medical Care After Burn Injury. *Medical Alphabet*. 2016;2(15(278)):6–12. (in Russ.)
3. Alekseev AA, Bobrovnikov AE, Bogdanov VV. Meaning of Innovative Technologies for Improvement of Results in Treating Burned Patients. *Medical Alphabet*. 2020;(13):44–47. (in Russ.) https://doi.org/10.33667/2078-5631-2020-13-44-47
4. Leung JJ, Fish J. Skin Grafts. *Univ Toronto Med J*. 2009;86(2):61–64.
5. Meek CP. Successful microdermagrafting using the Meek-Wall microdermatome. *Am J Surg*. 1958;96(4):557–558. PMID: 13571547 https://doi.org/10.1016/0002-9610(58)90975-9
6. Kamolz LP, Schintler M, Parvizi D, Selig H, Lumenta DB. The real expansion rate of meshers and micrografts: things we should keep in mind. *Ann Burns Fire Disasters*. 2013;26(1):26–29. PMID: 23966895
7. Cleland H, Wasiak J, Dobson H, Paul M, Pratt G, Paul E, et al. Clinical application and viability of cryopreserved cadaveric skin allografts in severe burn: a retrospective analysis. *Burns*. 2014;40(1):61–66. PMID: 24018216 https://doi.org/10.1016/j.burns.2013.05.006
8. Pianigiani E, Ierardi F, Cherubini Di Simplicio F, Andreassi A. Skin bank organization. *Clin Dermatol*. 2005;23(4):353–356. PMID: 16023930 https://doi.org/10.1016/j.clindermatol.2004.07.016
9. Shen ZA. Application of allogeneic skin in burn surgery. *Zhonghua Shao Shang Za Zhi*. 2019;35(4):243–247. PMID: 31060170 https://doi.org/10.3760/cma.j.issn.1009-2587.2019.04.002
10. Tanaka H, Aoki D, Shimazaki S. Skin allograft and skin bank. *Nihon Geka Gakkai Zasshi*. 2005;106(12):755–759. PMID: 16869130
11. Keswani SM, Mishra MG, Karnik S, Dutta S, Mishra M, Panda S, et al. Skin banking at a regional burns centre – The way forward. *Burns*. 2018;44(4):870–876. PMID: 29661552 https://doi.org/10.1016/j.burns.2017.11.010
12. Tavousi SH, Ahmadabadi A, Sedaghat A, Khaleghi E, Rashchi M, Bonakdaran Z. Skin allograft procurement and transplantation in Mashhad, Iran: Are burn patients' needs being met? *Cell Tissue Bank*. 2017;18(3):397–402. PMID: 28439732 https://doi.org/10.1007/s10561-017-9626-5
13. Kitala D, Kawecki M, Klama-Baryła A, Łabuś W, Kraut M, Glik J, et al. Allogeneic vs. Autologous Skin Grafts in the Therapy of Patients with Burn Injuries: A Restrospective, Open-label Clinical Study with Pair Matching. *Adv Clin Exp Med*. 2016;25(5):923–929. PMID: 28028957 https://doi.org/10.17219/acem/61961
14. Pleshkov AS. Development of allograft skin for wound coverage (Review of the literature). *Medico-Biological and Socio-Psychological Problems of Safety in Emergency Situations*. 2016;(2):34–46. (in Russ.) https://doi.org/10.25016/2541-7487-2016-0-2-34-46
15. Izmailov GA, Izmailov SG, Popov AN. Kombinierte autoallodermoplastik: klinische begrundungihrer anwendung. *Zeitschrift Dermatologie Deren Grenzgebiete*. 1996;182(1):13–14; 16.
16. Sachkov AV, Borovkova NV, Zhirkova EA, Mironov AS, Borisov VS, Spiridonova TG, et al. Use of cadaver skin in the treatment of wounds. *The Russian Journal of Transplantation*. 2018;10(4):327–335. https://doi.org/10.23873/2074-0506-2018-10-4-327-335

17. Wang C, Zhang F, Lineaweaver WC. Clinical Applications of Allograft Skin in Burn Care. *Ann Plast Surg*. 2020;84(3):158–160. PMID: 32028339 https://doi.org/10.1097/SAP.0000000000002282
18. Makarova ME, Borisov VS, Sachkov AV, Kaplunova MYu. Primenenie gidrogeley v praktike khirurga priemnogo otdeleniya ozhogovogo tsentra. *Moskovskaya meditsina.* 2019;(4):73. (in Russ.)
19. Pan Z, Ye H, Wu D. Recent advances on polymeric hydrogels as wound dressings. *APL Bioeng*. 2021;5(1):011504. PMID: 33644627 https://doi.org/10.1063/5.0038364 eCollection 2021 Mar.
20. Karyakin NN, Klemenova IA, Luzan AS. The outcomes of lower extremities burn wounds management by using of controlled moist environment. *Pirogov Russian Journal of Surgery*. 2017;(7):40–43. (in Russ.). https://doi.org/10.17116/hirurgia2017740-43
21. Pang C, Ibrahim A, Bulstrode NW, Ferretti P. An overview of the therapeutic potential of regenerative medicine in cutaneous wound healing. *Int Wound J*. 2017;14(3):450–459. PMID: 28261962 https://doi.org/10.1111/iwj.12735
22. Tottoli EM, Dorati R, Genta I, Chiesa E, Pisani S, Conti B. Skin Wound Healing Process and New Emerging Technologies for Skin Wound Care and Regeneration. *Pharmaceutics*. 2020;12(8):735. PMID: 32764269 https://doi.org/10.3390/pharmaceutics12080735
23. Zhang L, Yin H, Lei X, Johnson N, Lau Y, Yuan M, et al. A Systematic Review and Meta-Analysis of Clinical Effectiveness and Safety of Hydrogel Dressings in the Management of Skin Wounds. *Front Bioeng Biotechnol*. 2019;7:342. PMID: 31824935 https://doi.org/10.3389/fbioe.2019.00342 eCollection 2019.
24. Beschastnov VV, Ryabkov MG, Leontiev AE, Tulupov AA, Yudanova TN, Shirokova IYu, et al. Viability of Bacteriophages in the Complex Hydrogel Wound Dressings in vitro. *Modern Technologies in Medicine*. 2021;13(2):20–27. (in Russ.) https://doi.org/10.17691/stm2021.13.2.0
25. Kaur P, Gondil VS, Chhibber S. A novel wound dressing consisting of PVA-SA hybrid hydrogel membrane for topical delivery of bacteriophages and antibiotics. *Int J Pharm*. 2019;572:118779. PMID: 31740093 https://doi.org/10.1016/j.ijpharm.2019.118779
26. Gladkova ND, Petrova GA, Nikulin NK, Radenska-Lopovok SG, Snopova LB, Chumakov YuP, et al. In vivo optical coherence tomography imaging of human skin: norm and pathology. *Skin Res Technol*. 2001;6(1):6–16. PMID: 11428936 https://doi.org/10.1034/j.1600-0846.2000.006001006.x
27. Petrova GA, Petrova KS, Nemirova SV, Potekaev NN, Ksenofontov SYu, Vasilenkova TV. *3D-opticheskaya kogerentnaya tomografiya: prizhiznennaya otsenka morfologicheskikh osobennostey zdorovoy kozhi i patomorfologicheskaya diagnostika dermatozov*. Nizhniy Novgorod: IP Yakushov Yu.I. Publ.; 2018. (in Russ.)
28. Petrova GA, Petrova KS, Nemirova SV, Karpenko AA. 3D optical coherence tomography: possibilities in the diagnosing skin neoplasms. *Klinicheskaya Dermatologiya i Venerologiya*. 2019;18(6):715–727. (In Russ.). https://doi.org/10.17116/klinderma201918061715
29. Deegan AJ, Lu J, Sharma R, Mandell SP, Wang RK. Imaging human skin autograft integration with optical coherence tomography. *Quant Imaging Med Surg*. 2021;11(2):784–796. PMID: 33532277 https://doi.org/10.21037/qims-20-750

Received on 08.08.2021

Review completed on 07.09.2021
Accepted on 27.12.2021