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ANNOTATION
OF PHD DOCTORAL DISSERTATION

Dissertation topic: Combined Methods for Correcting Wound Healing in Chronic Non-Healing Wounds in Patients with Chronic Obliterative Arterial Disease of the Lower Extremities

Educational Program: 8D10102 "Medicine"
Doctoral Candidate: Azat Chinaliyev
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Scientific advisors: professor, dr. sci. (med.) Bazylbek Zhakiev
associate professor, PhD Samat Saparbayev
professor, dr. med. Tokan Sultanaliyev
dr. sci. (med.) Evgeny Kretov (Russia)
PhD, professor Luis René Arias Villarroel (Mexico)

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1. Research Relevance. Chronic obliterative diseases of the lower extremity arteries, or peripheral arterial disease (PAD), represent one of the most common manifestations of systemic atherosclerosis [1, 2]. These conditions are characterized by progressive stenosis or occlusion of lower extremity arteries and are accompanied by impaired tissue perfusion, leading to the development of ischemia and formation of chronic non-healing wounds. According to literature data, PAD is diagnosed in approximately 202 million people worldwide [3], and its prevalence increases with age and the accumulation of risk factors: diabetes mellitus (DM), arterial hypertension (AH), hypercholesterolemia, smoking, as well as the presence of a burdened family history [4, 5].

In Kazakhstan, according to data from the National Scientific Center for Healthcare Development named after Salidat Kairbek, Ministry of Health of the Republic of Kazakhstan, more than 6,000 patients annually undergo surgical treatment for obliterative atherosclerosis of extremity arteries, and in more than 400 cases the disease results in fatal outcome.

In one-third of cases, trophic wounds associated with chronic obliterative diseases of the lower extremity arteries do not heal over prolonged periods, and 70% of patients experience recurrences, which significantly reduces quality of life and leads to high medical and social costs [6].

In economically developed countries, billions of dollars are spent annually on the treatment of chronic non-healing lower extremity wounds. Thus, in the United States, these expenditures amount to approximately $22.5 billion per year. Only 50–75% of patients with chronic wounds achieve healing within 6 months with standard therapy, which underscores the limited effectiveness of existing methods [7, 8].

Chronic non-healing wounds have become a "silent epidemic" affecting more than 40 million people worldwide, impairing quality of life and leading to disability and premature mortality. Mean life expectancy following lower extremity amputation is approximately 3 years, and five-year survival does not exceed 40% [9, 10].

Based on the pathogenic principles of chronic non-healing wounds of the extremities, successful treatment is impossible without improvement of circulation and elimination of hemodynamic disturbances in the affected extremity; unfortunately, this alone is insufficient for complete and rapid wound healing. In the combination of treatment methods for chronic non-healing wounds of the extremities in the setting of chronic obliterative diseases of the lower extremity arteries, surgical operations represent the leading component. Traditional open reconstructive operations are associated with high risk of complications, particularly in elderly and medically complex patients. For this reason, endovascular revascularization is the method of choice, owing to its minimal invasiveness, high safety profile, and accessibility [11].

However, even with successful revascularization, achieving complete and stable healing of trophic wounds is not always possible. This indicates the need for comprehensive treatment utilizing combined methods that include not only restoration of blood flow but also stimulation of tissue regenerative processes [12, 13].

One of the promising directions in modern regenerative medicine is cellular therapy. Fibroblasts—connective tissue cells actively participating in reparative regeneration, synthesis of extracellular matrix, collagen, elastin, and hyaluronic acid, as well as in epithelialization and angiogenesis—are of particular interest [14, 15]. Their application in the treatment of non-healing wounds can significantly accelerate healing and improve the quality of the newly formed tissue. Fibroblasts possess the ability to enhance cell proliferation and differentiation, as well as mobilize the phagocytic response, which is especially important in the setting of chronic ischemia and metabolic disturbances associated with DM [16, 17].

Thus, despite significant advances in vascular surgery and regenerative medicine, the problem of effective treatment in this patient population remains relevant. Currently, there are no unified and standardized approaches ensuring reliable and complete healing of ischemic wounds, which necessitates comprehensive research aimed at optimizing therapeutic strategies and

developing effective methods for wound healing correction. The search for solutions to the aforementioned issues formed the foundation for our scientific investigations.

2. Objective

To evaluate the effectiveness of a combined method for wound healing correction through endovascular revascularization and local application of allogeneic fibroblasts in chronic non-healing wounds in patients with chronic obliterative arterial disease of the lower extremities.

3. Research Tasks

1. Develop and justify a combined treatment method for chronic non-healing wounds in chronic obliterative arterial diseases of the lower extremities, combining local application of allogeneic fibroblasts with endovascular revascularization.
2. Study the course of regenerative processes in chronic non-healing lower extremity wounds when combining local application of allogeneic fibroblasts with endovascular revascularization in chronic arterial insufficiency.
3. Evaluate immediate and long-term results of the developed combined treatment method for chronic non-healing lower extremity wounds compared to endovascular revascularization alone without allogeneic fibroblasts.

4. Materials and Methods

Study Design: Randomized controlled trial

Randomization: Patient randomization was performed using the RandStuff online random number generator (hospital record number in KMIS [Comprehensive Medical Information System] corresponded to patient number).

Study Subjects:

The study population consisted of 116 patients with chronic non-healing wounds secondary to chronic obliterative arterial disease of the lower extremities. All patients were allocated into two groups based on the treatment method employed:

Study Group — 58 patients with chronic obliterative arterial disease of the lower extremities and chronic non-healing wounds who underwent endovascular revascularization combined with local application of allogeneic fibroblasts.

Control Group — 58 patients who also underwent endovascular revascularization; however, conventional surgical debridement methods were used during the postoperative period.

Inclusion Criteria:

1. Male and female patients aged 18–85 years inclusive.
2. Patients with a diagnosis of PAD (ICD-10 codes I70.2–I70.9) confirmed by results of ultrasound duplex scanning of vessels.
3. Patients with chronic non-healing wound (duration >1 month) and intermittent claudication with pain-free walking distance less than 200 meters.

Exclusion Criteria:

1. Acute myocardial infarction (ACS), acute cerebrovascular accident (ACS-stroke), pulmonary embolism (PE).
2. Acute infectious diseases.
3. Patients who underwent reconstructive intervention on lower extremity arteries (surgical or endovascular) within 3 months prior to randomization.
4. Patients with history of hypersensitivity or allergy to radiographic contrast agent, similar medications, or excipients. Complicated allergic history to reagents and reactants used during treatment.

5. Pregnant or breastfeeding patients, or women planning to become pregnant before or during study participation, or intending to become egg donors during this same period.
6. Presence of peptic ulcer disease of the stomach and duodenum (DU).
7. History of benign or malignant neoplasms.

Research Methods

Instrumental Investigation Methods

Multidetector computed tomography with contrast (MDCT) was used to assess vascular anatomy, structure, and extent of the pathological process.

Duplex ultrasound (DUSG) with ankle-brachial index (ABI) measurement using Sono Scape S6Pro apparatus was performed for preliminary blood flow assessment before and after surgical intervention, at 1, 3, 6, and 12 months.

Assessment of microcirculatory changes in the lower extremities was performed by measuring transcutaneous oxygen tension in tissues ($TcpO_2$) before and after surgical intervention, at 1, 3, 6, and 12 months using TCM TOSCA monitor for continuous transcutaneous $TcpO_2$ measurement with Sensor 92 transcutaneous probe.

Pain intensity in the lower extremities was assessed using the "Visual Analog Pain Scale" recommended and presented in 2017 by the International Association for the Study of Pain.

For objective and quantitative assessment of chronic non-healing wound dimensions, wound area measurement was performed. Initial measurement was conducted using a standard centimeter ruler with clearly visible graduations and numbers. Subsequently, the wound was covered with sterile, transparent medical polyethylene film used in surgical practice for wound dressing fixation; wound contours were traced along the perimeter with photographic documentation (digital photo documentation), followed by analysis using the specialized mobile application Lesion Meter, which automatically calculates wound area and displays results on screen. Wound volume was calculated in square centimeters.

Bacteriological Investigation

To study the microbiota of chronic non-healing wounds, wound exudate was collected using two swabs following preliminary wound cleansing and debris removal. One swab was moistened with sterile physiological saline for microscopic examination, while the other was used for culture.

Morphological Investigation

Histological examination of wound biopsies was performed using standard methodology in the Department of Pathomorphology at the National Scientific Oncology Center. Review of histological glass slides with detailed scientific description was conducted at the Department of Histology under the supervision of MD Zhanna Evgenievna Komekbai, West Kazakhstan Marat Ospanov Medical University. Microscopic examination of histological specimens was performed using a light microscope at magnifications of ×40; ×100; ×400 using the AxioLab A1 digital light microscope (registration certificate RK-MT-7№009046, manufactured in Germany, date of state registration: August 17, 2018). Biopsies were obtained at baseline, and at 7, 14, 30, and 90 days following treatment initiation.

Additionally, all patients upon admission were prescribed comprehensive therapy, and when necessary, minor surgical procedures were performed for drainage of foot abscesses and phlegmons, necrotomy, and exarticulation. Specialist consultations were obtained as indicated.

Endovascular Revascularization Technique

In the fluoroscopic angiography operating room: following operative field preparation under local anesthesia with 0.5% Novocaine solution or 0.2% Lidocaine 10 mL, anterograde or retrograde puncture and catheterization of the appropriate common femoral artery was performed using the Seldinger technique. A 6Fr introducer was placed in the arterial lumen through which diagnostic catheters were advanced to the area of interest. Subsequently, radiographic contrast

agent was administered intra-arterially and a series of diagnostic images were obtained in real-time, based on which the degree and character of vascular involvement was determined. A decision was then made regarding recanalization via percutaneous transluminal balloon angioplasty or stenting of the affected segment. In cases of vessel occlusion, recanalization was performed as the first step, followed by restoration of vessel patency.

A balloon or stent of appropriate size (according to the native diameter of the affected vessel) was delivered to the lesion zone, sequential balloon dilation was performed with inflation duration up to 5 minutes, or vessel stenting was performed.

Following completion, control angiography was conducted to assess the obtained result.

Technique of Local Application of Allogeneic Fibroblasts

Procurement, cultivation, and cryopreservation of allogeneic fibroblasts was performed at the laboratory of National Biotechnology Center, Ltd., Astana. The source of allogeneic fibroblasts is a transferrable cell culture obtained from neonatal foreskin (Patent #27238 dated August 15, 2013). Within the scope of medical services delivery, the research center procures prepared diploid fibroblast culture with provision of a biological passport certifying sterility and the percentage of viable cells in the supplied batch.

In clinical practice, the use of allogeneic fibroblasts in patients is regulated by clinical protocol code 86.66 "Allotransplantation of skin (diploid fibroblast culture)" approved by Order of the Minister of Health of the Republic of Kazakhstan dated October 16, 2020 № КР ДСМ-134/2020 and registered with the Ministry of Justice of the Republic of Kazakhstan on October 21, 2020 under № 21471.

Fibroblast transportation is conducted in accordance with the Order of the Minister of Health of the Republic of Kazakhstan dated November 25, 2020 № КР ДСМ-207/2020, registered with the Ministry of Justice of the Republic of Kazakhstan on November 27, 2020 under № 21683, "On approval of rules and conditions for procurement, storage, preservation, transportation, and transplantation of organs (parts of organs) and/or tissues (parts of tissues) from donor to recipient."

Prepared allogeneic fibroblasts contained in sterile containers were used within 2–4 hours following preparation to maintain optimal viability. Application was performed via aerosol spray at a distance of 10–15 cm, uniformly covering the entire wound surface defect area, applied twice at 2-hour intervals. Each container contained 10 mL of 0.9% NaCl solution and cell suspension at a concentration of 2×10^6 fibroblasts/mL, sufficient for coverage of the wound surface.

Statistical Methods:

Data collection, systematization of primary information, and database formation were performed using MS Excel 2021. Statistical analysis and graphical presentation of study results were performed using STATA 19.5 software (StataCorp LLC, Texas 77845–4512, USA).

Descriptive statistical methods were used to describe the obtained data. In cases of normal data distribution, mean value (M), standard deviation, and standard error of the mean (m) were calculated. These indicators allow assessment of the precision of the mean value. If data did not demonstrate normal distribution, median (Me) and interquartile range (IQR) were used. The median reflects the "average" value in the sample, while IQR reflects the spread of 50% of central values (25th–75th quartiles).

Normality of distribution of quantitative variables was verified using graphs and the Shapiro–Wilk test. For comparison between two independent groups, the classical t-test was employed. The chi-square (χ^2) test was applied for categorical variables. Baseline ulcer size demonstrated pronounced asymmetry, therefore median with interquartile range (IQR) and the nonparametric Mann–Whitney test were used.

5. Study Results

All operations were performed within the framework of the guaranteed volume of free medical care (GVFMC).

Treatment commenced with wound revision and surgical debridement, followed by conservative therapy including antibacterial and vasodilatory agents. Following control of the inflammatory process, endovascular revascularization for ischemia correction was performed.

In the study group, allogeneic fibroblasts were applied locally following wound treatment, while in the control group, conventional treatment methods were employed.

Statistical analysis between the study and control groups revealed no statistically significant differences in the majority of baseline characteristics. Mean age in the study group was 55.7 ± SD years, and in the control group 57.3 ± SD years ($p = 0.06$).

Gender distribution showed no statistically significant differences ($p = 0.6$), with male predominance in both groups: 43 in the study group and 37 in the control group; female distribution was 15 in the study group and 21 in the control group.

Mean BMI values were similar in the study group (27.4 ± SD) and control group (27.7 ± SD) ($p = 0.45$). The number of smoking patients in the study group was 28 patients, and in the control group 24 patients ($p = 0.6$).

Regarding comorbidities: the proportion of participants with diabetes in the study group was 24 patients and in the control group 36 patients ($p = 0.2$); patients with arterial hypertension—25 (43.1%) in the study group versus 22 (37.9%) in the control group ($p = 0.7$); renal insufficiency—11 (19%) patients in the study group versus 8 (13.8%) in the control group ($p = 0.5$); coronary artery disease (CAD)—6 (10.3%) in the study group and 3 (5.2%) in the control group ($p = 0.3$); patients with hypertension and diabetes—8 (13.8%) in the study group and 4 (6.9%) in the control group ($p = 0.3$); and patients with multiple diseases—4 (6.9%) in the study group and 3 (5.2%) in the control group ($p = 0.7$). These showed no statistically significant differences between groups despite slight numerical predominance in the study group. Additionally, the proportion of smoking patients in the study group was 28 (48.3%) and in the control group 24 (41.4%), $p = 0.6$.

Analysis of chronic lower extremity ischemia severity according to the Rutherford classification, proposed in the 2020 recommendations of the European Society of Vascular Surgeons, demonstrated comparable patient distribution between the study groups.

Grade I ischemia predominated in the patient population. The largest group consisted of patients with Category 2 ischemia: 23 (39.7%) patients in the study group versus 29 (50%) patients in the control group ($p = 0.5$). Patients with Category 3 ischemia (rest pain) comprised 12 (20.7%) patients in the study group versus 14 (24.2%) patients in the control group ($p = 0.7$).

Grade II ischemia (Rutherford Category 4—minor ulcerative defects) was diagnosed in 10 (17.2%) patients in the study group versus 6 (10.3%) patients in the control group ($p = 0.3$).

The most severe Grade III ischemia (Category 5—extensive ulcerative defects or gangrene) was identified in 13 (22.4%) patients in the study group versus 9 (15.5%) patients in the control group ($p = 0.4$).

Statistical analysis revealed no significant differences in patient distribution by ischemia severity grade between groups (all p values > 0.05), demonstrating comparable baseline disease severity between groups.

In patient distribution by wound severity grade (Wound), the obtained value $\chi^2 = 0.14$ with p value > 0.05 indicated absence of statistically significant differences in wound severity distribution between groups. This indicated comparability of wound severity indicators in both groups.

Detailed analysis revealed the following distribution of wound severity grades:

Grade I (wound ≤3 cm): Grade I wounds were observed in 26 patients in the study group and 24 patients in the control group ($p = 0.718$).

Grade II (wound ≤5 cm): Grade II wounds were observed in 32 patients in the study group and 34 patients in the control group ($p = 0.718$).

Clinical patient distribution by type of microcirculation impairment (Ischemia) based on ABI indicators: in the study group, the number of patients with moderate lower extremity ischemia was 13 (22.4%) patients, and in the control group this indicator was 12 (20.7%) patients. The majority in the compared groups consisted of patients with critical lower extremity ischemia, corresponding to 27 (46.5%) patients in the study group and 25 (43.1%) patients in the control group. Severe extremity ischemia was identified in 18 (31.03%) patients in the study group and 21 (36.2%) in the control group.

The overall value $\chi^2 = 0.346$ with $p > 0.05$ indicated absence of statistically significant differences in patient distribution by ABI grades between the study groups, demonstrating group comparability for this indicator.

In analysis of patient distribution by degree of ischemic involvement, between the study group (n = 58) and control group (n = 58), Grade 0 involvement was not detected, reflecting absence of normal microcirculation parameters in patients. The number of patients with Grade 1 involvement ($TcpO_2$ 40–59 mmHg) in the study group comprised 60.3% (n = 35), in the control group 55.2% (n = 32), where p = 0.657, with no statistically significant differences established between compared groups. With Grade 2 involvement (30–39 mmHg)—34.5% (n = 20) in the study group and 36.2% (n = 21) in the control group (p = 0.855), indicating absence of intergroup differences. At Grade 3 involvement (<30 mmHg), more severe perfusion disturbances occurred in 5.2% (n = 3) of study group patients and 8.6% (n = 5) of control group patients (p = 0.464). No statistically significant differences were identified between the study and control groups in patient distribution by transcutaneous oxygen tension levels ($p > 0.05$). $TcpO_2$ parameters in both groups were comparable.

Regarding degree of chronic non-healing wound infection, patients with Grade 1 infection were most frequently encountered: in the study group the number of patients comprised 31 (53.4%) and in the control group 32 (55.2%), where p = 0.857. Patients without signs of infection numbered 19 (32.6%) and 18 (31.03%) respectively, with p = 0.873. The number of patients with deep tissue infection (tendons) in the study group was 5 (8.6%) and in the control group 6 (10.3%), p = 0.754. Patients with extensive infection, corresponding to Grade 3 according to WIFI classification, numbered 3 (5.2%) in the study group and 2 (3.45%) in the control group.

The obtained results revealed no statistically significant differences between groups ($\chi^2 = 4.10$, p = 0.250), indicating sample homogeneity for this indicator.

Analysis of atherosclerotic lesion localization distribution of lower extremity arteries revealed absence of statistically significant differences between the study and control groups. In segmental involvement, tibial artery involvement was most frequently encountered in 12 (20.7%) patients in the study group and 10 (17.2%) in the control group (p = 0.674), and popliteal artery involvement in 7 (12.06%) and 5 (8.6%) respectively (p = 0.553). Aortoiliac segment and common femoral artery involvement was observed less frequently and comprised 1 (1.7%) patient in the study group and 2 (3.4%) in the control group (p = 0.559), as well as 3 (5.2%) and 4 (6.9%), p = 0.708 respectively. Among multisegmental involvement, combined popliteal and tibial artery involvement predominated in 17 (29.3%) patients in the study group and 15 (25.9%) in the control group (p = 0.687). Combined femoral and tibial artery involvement occurred in 11 (18.9%) patients in the study group versus 13 (22.4%) in the control group, p = 0.662. Combined iliac and femoral artery involvement was observed in 7 (12.06%) versus 9 (15.5%) patients respectively (p = 0.652).

The comparative analysis conducted of baseline clinical-functional characteristic parameters of patients in the study and control groups demonstrated their complete comparability, ensuring correctness of subsequent comparison of treatment method effectiveness.

The most objective assessment of any treatment method is its results. Therefore, the effectiveness of treatment in patients with chronic non-healing wounds in chronic obliterative arterial disease of the lower extremities was evaluated based on dynamics of regional oxygenation, pain syndrome, wound healing, treatment duration, and treatment outcomes.

All patients tolerated surgical intervention well and, on the first postoperative day, reported improvement in general condition and resolution of lower extremity pain, as well as marked

increase in limb warmth. This was attributable to successful recanalization and restoration of blood flow in the lower extremities. During subsequent dynamic follow-up, duplex ultrasound with ABI measurement was performed in patients; in both groups the indicators improved substantially: from approximately 0.4 preoperatively to approximately 0.9 postoperatively, maintaining a level of approximately 1.0 throughout the observation period. No substantial differences were observed between groups (statistically significant ABI differences were not observed between groups at any time point [all $p > 0.05$]). This demonstrates the effectiveness of revascularization in both groups and indicates that additional fibroblast therapy did not exert additional influence on ABI.

Assessment of transcutaneous partial oxygen pressure revealed predictable improvement in tissue oxygenation following revascularization in both groups: from baseline 40.6–40.7 mmHg to 59.1–59.6 mmHg in the early postoperative period with subsequent progressive increase to 90.5–92.2 mmHg by 12 months. Baseline $TcpO_2$ in both groups was approximately 40%. Postoperatively, values increased to approximately 60% and reached >90% by 12 months. Thus, fibroblast therapy also did not result in improved oxygenation, and the observed late improvement in ulcer healing should be explained by local tissue effects rather than systemic perfusion.

Analysis of ABI and $TcpO_2$ measurements was performed using a linear mixed-effects model; higher values indicate better perfusion.

The obtained data demonstrate that application of allogeneic fibroblasts does not exert significant influence on macrohaemodynamic parameters and regional oxygenation indicators. Consequently, the therapeutic effect of cellular therapy should be considered in the context of local wound process effects mediated by paracrine mechanisms, stimulation of reparative processes, and modulation of the wound microenvironment, which required further investigation of direct indicators of ulcerative defect healing.

Comparison of chronic wound healing timeline between the study group with local allogeneic fibroblast application and the control group with standard wound management based on healing stage.

Initial healing. In the allogeneic fibroblast group, median time to initial healing was approximately 2.5 months (range 2–4 months), which was substantially less compared to the control group, where median was approximately 3.5 months (range 3–4.5 months). The difference was statistically significant and demonstrates acceleration of the healing process with allogeneic fibroblast use.

Complete healing. More pronounced differences were observed in the timeframe for complete ulcerative defect healing. In the study group, median time to complete healing was approximately 5 months (range 4.5–6 months), whereas in the control group it was approximately 6 months (range 5–6.5 months).

Application of allogeneic fibroblasts resulted in reduction of both initial healing duration by 1 month and complete healing duration by 1 month compared to standard methodology.

Correlation analysis results confirm the clinical advantage of allogeneic fibroblast use in accelerating chronic wound healing following endovascular interventions. Data analysis demonstrates a clear bimodal pattern of treatment results. An ascending trend line indicates positive correlation between observation duration and healing degree, which may reflect the cumulative therapeutic effect of the innovative approach using cellular technologies.

Confidence intervals for each time point demonstrate statistical significance of the obtained differences between groups, confirming clinical significance of allogeneic fibroblast application in comprehensive treatment of chronic ulcerative lesions.

Thus, local application of allogeneic fibroblasts combined with endovascular revascularization resulted in reduction of both initial healing duration from 3.5 months to 2.5 months and complete healing of chronic non-healing wounds by 1 month (from 6 to 5 months) compared to standard methodology, which is confirmed by correlation analysis results.

Comparative analysis of pain syndrome dynamics, assessed using Visual Analog Scale (VAS), revealed varying pain intensity in the study and control groups at different observation stages. Prior to surgical intervention, mean VAS values showed no statistically significant

differences and comprised 6.46 ± 1.50 points in the study group and 6.84 ± 1.62 points in the control group ($p = 0.196$), indicating baseline comparability of pain syndrome severity.

Twenty-four hours following surgery, pain level remained equally high in both groups: 6.39 ± 1.49 points versus 6.78 ± 1.56 points respectively ($p = 0.175$). Statistically significant differences began to manifest at the time of discharge, when pain intensity in the study group decreased to 5.68 ± 1.55 points, whereas in the control group it remained at 6.43 ± 1.77 points ($p = 0.016$).

Three months following surgery, differences became more pronounced: VAS scores were 4.38 ± 1.81 points in the study group versus 5.26 ± 1.82 points in the control group ($p = 0.010$). Similar tendency persisted at 6 months, where scale values were 4.27 ± 1.91 and 5.36 ± 1.99 points respectively ($p = 0.003$).

Twelve months following intervention, further reduction in pain syndrome severity was observed in both groups; however, statistically significant differences between them were no longer noted: 2.79 ± 1.47 points in the study group and 3.09 ± 1.65 points in the control group ($p = 0.305$).

Results of comparative pain syndrome dynamics analysis demonstrate that pain intensity in the area of chronic non-healing wounds was considerably lower in the patient group where endovascular revascularization was combined with local allogeneic fibroblast application beginning from the time of discharge (5.68 ± 1.55 versus 6.43 ± 1.77 points, $p = 0.016$).

In examining the dynamics of chronic non-healing wound size changes during observation, baseline wound size was 8.7 cm^2 in the study group and 6.8 cm^2 in the control group ($p = 0.17$). One month later, mean wound size decreased in both groups without substantial intergroup difference. Beginning at three months, the study group demonstrated marked reduction: at 3 months, mean wound size was 0.9 cm^2 compared to 3.3 cm^2 in the control group ($p < 0.001$); at 6 months—0.4 versus 3.5 cm^2 ($p < 0.001$); and at 12 months—near-complete healing in patients with allogeneic fibroblasts (0.1 cm^2) compared to chronic non-healing wounds in the control group (3.5 cm^2, $p < 0.001$). These descriptive longitudinal results (mean values and p values at each time point) provide initial impression of treatment effect but do not account for baseline differences or patient correlations. Analysis was performed using the independent samples t-test.

Treatment effectiveness was also assessed based on wound healing dynamics and treatment outcomes.

During wound cleansing and healing, all phases progress sequentially—inflammatory phase, proliferative phase, and scar remodeling phase.

During research execution and in fulfillment of the second objective, histological changes following fibroblast transplantation were analyzed, demonstrating characteristic dynamics of integration and functional activity of transplanted cells in recipient tissues.

Wound healing in chronic obliterative arterial disease is characterized by prolonged inflammatory phase and inhibited proliferation. To detect these changes and evaluate the effect of allogeneic fibroblast application, wound biopsy microspecimens were examined. Biopsies were obtained at baseline and at 7, 14, 30, and 90 days following treatment initiation.

In the study group at day 7 was observed: marked inflammatory reaction (lympho-histiocytic infiltration with neutrophils), granulation tissue areas with active fibroblast proliferation, sprouting capillaries, early epithelialization; at day 14: mature granulation tissue, intensive matrix and collagen synthesis, reduced inflammation, marked neoangiogenesis. At day 30: active epithelialization, multilayered epithelium formation, remodeling processes predominate. At day 90: complete epithelialization, fibroblast integration with formation of organized collagen bundles, vascular network reduction—completion of repair. In the control group: at day 7: tissue debris, massive neutrophilic infiltration with dystrophy signs. At day 14: debris lysis, persistent polymorphic infiltration, absence of mature granulation and collagen formation. At day 30: moderate lympho-histiocytic infiltration, residual debris, absence of mature granulation, slowed repair.

At day 90: chronic inflammation, incomplete epithelialization, thin loose collagen bundles without complete scarring, incomplete neoangiogenesis, focal areas of residual tissue debris and

dystrophically altered cellular elements indicating protracted inflammatory-destructive processes and delayed regeneration.

Comparative analysis of morphological data from the study and control groups demonstrated that local application of allogeneic fibroblasts combined with endovascular revascularization facilitates optimization of the phased wound healing process, accelerated transition from inflammation to proliferation and remodeling, as well as enhanced efficacy of reparative regeneration.

Model-based predicted wound healing probabilities: for initial healing, predicted probability steadily increased to 93.7% in the study group at 12 months compared to 47% in the control group. For complete healing in study group patients, probability again reached 93.7%, whereas in the control group it remained at only 33%.

Kaplan-Meier analysis illustrates cumulative probabilities of initial and sustained wound healing, calculated by time to event occurrence, accounting for censoring.

Initial healing: by 12 months, initial healing was achieved in nearly all patients in the study group (~94%) compared to less than half of patients in the control group (~47%).

Complete healing: the difference proved even more striking. By 12 months, 94% of patients who received cellular therapy (allogeneic fibroblasts) maintained complete healing, whereas in the control group this rate was only 33%.

Thus, Kaplan-Meier analysis is important because it provides insight into wound healing from a survival perspective, reflecting not only "whether healing occurs" but also "when." It demonstrates earlier and more reliable healing in the study group.

Analysis of treatment outcomes was conducted based on frequency of repeat surgical interventions, number of minor foot amputations, and number of major limb amputations.

In analysis of treatment outcomes in the patient group where combined treatment was applied, reduction was identified in frequency of repeat surgical interventions from 41.4% to 22.4%—approximately two-fold reduction—and in major limb amputations from 12.1% to 3.4%—a 3.5-fold reduction ($p = 0.047$).

In analysis of hospitalization duration, patients in both the study and control groups were hospitalized for 5.3 ± 1.2 hospital days, indicating low invasiveness of this treatment method not requiring prolonged rehabilitation. Subsequently, patients underwent outpatient treatment, where study group patients were observed for 58.6 ± 3.4 days, and control group patients for 81.2 ± 1.8 days; the difference in treatment duration between the study and control groups was statistically significant ($p < 0.001$).

The obtained immediate results demonstrate that the combined treatment method through endovascular revascularization with local allogeneic fibroblast application is an effective treatment approach.

In assessment of treatment results in the compared groups in the long-term period, it was identified that following application of combined treatment for chronic non-healing lower extremity wounds, good results were maintained in 40 (69%) patients, whereas in the control group only 19 (32.7%). Satisfactory treatment results were noted in 14 (24.1%) patients in the study group and 23 (39.7%) patients in the control group.

Unsatisfactory results were observed most frequently in the control group following conservative treatment application in 16 (27.6%) patients and in 4 (6.9%) patients in the study group ($p < 0.001$).

6. Scientific Novelty

1. For the first time, a combined treatment method for chronic non-healing wounds in chronic obliterative arterial disease of the lower extremities has been developed and clinically implemented, incorporating endovascular revascularization combined with local application of allogeneic fibroblasts (Patent Application № 2025/0718.1 dated July 31, 2025).

2. For the first time, the characteristics of chronic non-healing lower extremity wounds during application of combined treatment, including local cellular therapy with allogeneic fibroblasts combined with endovascular revascularization, have been established, with the outcome being activation of reparative regeneration and acceleration of wound defect healing.
3. For the first time, clinical efficacy evaluation of the developed treatment method, based on the combination of local application of allogeneic fibroblasts and endovascular revascularization in chronic non-healing wounds in patients with chronic obliterative arterial disease of the lower extremities, has been conducted.

7. Theoretical and Practical Significance

- The theoretical significance of the conducted research lies in the scientific and clinical substantiation of the concept of combined wound healing correction based on the combined use of endovascular revascularization and allogeneic fibroblasts.
- A new combined treatment method for chronic non-healing wounds in chronic obliterative arterial disease of the lower extremities has been developed and implemented into clinical practice, performed through endovascular revascularization with local application of allogeneic fibroblasts, which expands the arsenal of surgical treatment methods.
- The developed combined treatment method allows reduction of repeat surgical interventions and major limb amputations, thereby improving treatment outcomes in patients with chronic non-healing lower extremity wounds secondary to chronic obliterative atherosclerosis of the lower extremity arteries.

8. Propositions for Defense

1. The combined method for correcting chronic non-healing wounds in patients with chronic obliterative arterial disease of the lower extremities, based on the combination of endovascular revascularization with local application of allogeneic fibroblasts, is pathogenetically justified, technically feasible, and clinically highly effective treatment approach for this patient category.
2. Local application of allogeneic fibroblasts combined with endovascular revascularization contributes to improvement of the wound healing process—accelerated transition from inflammation to proliferation and remodeling, as well as increased efficacy of reparative regeneration of the wound surface.
3. The combination of local application of allogeneic fibroblasts with endovascular revascularization in chronic non-healing wounds allows acceleration of both initial and complete wound healing, reduction of pain syndrome intensity, decrease in the number of repeat surgical interventions and major limb amputations, thereby reducing treatment duration and the number of patients with unsatisfactory outcomes.

9. Conclusions

Based on the results of the conducted research, the following conclusions have been formulated.

1. The developed combined treatment method for chronic non-healing wounds in patients with chronic obliterative arterial disease of the lower extremities, based on the combination of endovascular revascularization with local application of allogeneic fibroblasts, is a justified and clinically highly effective approach for wound healing correction in this pathology.
2. Local application of allogeneic fibroblasts combined with endovascular revascularization in the treatment of chronic non-healing lower extremity wounds substantially improves the course of local reparative processes, manifesting as shortened inflammatory phase, activation of tissue proliferation and remodeling, thereby contributing to four-fold wound reduction at 3 months (mean wound size 0.9 cm^2 compared to 3.3 cm^2 in the control group [$p < 0.001$]); nine-fold reduction at 6 months—0.4 cm^2 versus 3.5 cm^2 ($p < 0.001$); and at 12 months—near-complete healing (0.1 cm^2) compared to the control group 3.5 cm^2 ($p < 0.001$).

3. The combination of endovascular revascularization with local application of allogeneic fibroblasts in comprehensive treatment of chronic non-healing wounds in chronic obliterative arterial disease of the lower extremities reduces pain intensity from the time of discharge in the area of chronic non-healing wounds (5.68 ± 1.55 versus 6.43 ± 1.77 points, $p = 0.016$), decreases frequency of repeat surgical interventions from 41.4% to 22.4% and major limb amputations from 12.1% to 3.4% ($p = 0.047$), reduces treatment duration two-fold compared to the control group ($p < 0.05$).
4. Application of the developed combined treatment method for chronic non-healing wounds in chronic obliterative arterial disease of the lower extremities allows achieving good and satisfactory long-term results in 69% and 24.1% of patients respectively, and reduces the number of patients with unsatisfactory outcomes from 27.6% to 6.9% ($p < 0.001$).

10. Practical recommendations

1. In chronic non-healing wounds in patients with chronic obliterative arterial disease of the lower extremities, the preferred method may be the combination of endovascular revascularization with local application of allogeneic fibroblasts.
2. Allogeneic fibroblasts must be prepared in a specialized laboratory and used within 2–4 hours following preparation to maintain optimal cell viability.
3. Application of allogeneic fibroblasts should be performed using aerosol spray method on the cleaned wound surface at a distance of 10–15 cm, uniformly covering the entire defect area, applied twice at 2-hour intervals.
4. In comprehensive therapy, antiplatelet agents are mandatory for prevention of thrombotic complications following endovascular intervention.
5. Dynamic monitoring of treatment efficacy should be conducted with assessment of wound area, granulation tissue status, degree of epithelialization, and pain syndrome severity.

11. Implementation forms

Three scientific papers have been published on the topic of the dissertation, in Scopus, quartile 2. The research results have been published in two collections of abstracts.

1. "Femoro-popliteal endovascular interventions." Videosurgery Miniinv 2024; 19 (2): 187–197. DOI: https://doi.org/10.5114/wiitm.2024.139548

2. Journal with Q2 "European Review for Medical and Pharmacological Sciences"
Article title: "Results of endovascular interventions for peripheral arterial diseases on the targeted arterial segments." 2024 Oct; 28 (20): pp. 4451–4460.
DOI: 10.26355/eurrev_202410_36868.

3. Journal with Q2 "Wide Surgery and Infrared Techniques"
Article title: "Allogeneic fibroblasts versus conventional debridement after successful endovascular interventions on the healing of chronic ulcers following peripheral arterial diseases."
DOI: 10.20452/wiitm.2025.17959

4. Chinaliev A.M., Sultanaliev T.A., Zhakiev B.S., Kretov E.I., Saparbayev S.S., Khasenov D.T. "Combined methods of wound healing for long-term non-healing wounds in patients with chronic obliterating disease of the arteries of the lower extremities." Collection of abstracts of the 5th Congress of the Kazakhstan Society of Vascular Surgeons on the topic: "Selected issues of angiology and vascular surgery." "A Look into the Future" Turkestan, May 23-24, pp. 51-52.

5. Chinaliev A.M., Sultanaliev T.A., Zhakiev B.S., Kretov E.I., Saparbayev S.S., Luis R.A. "Combined Methods for Correcting Long-Term Non-Healing Wounds of the Lower Extremities." Proceedings of the IV Congress of the Kazakhstan Venous Forum with International Participation. August 9-10, 2024. Almaty, pp. 49-50.

Presentations at international conferences as speaker: USA (Miami – September 2023), Bolivia (Santa Cruz de la Sierra – December 2023), Uzbekistan (Tashkent) 2023, UAE (Dubai) 2024,

Russia (Moscow, St. Petersburg, Petrozavodsk) 2024, Denmark (Copenhagen), "V Congress of the Kazakh Society of Vascular Surgeons" May 23, 2025, Turkestan.

12. Results of research implementation in practical healthcare and educational process

1. The results of this research are being utilized in comprehensive treatment of patients with chronic non-healing wounds secondary to chronic obliterative arterial disease of the lower extremities in the vascular surgery department of the National Scientific Oncology Center, Astana.
2. Intellectual property – 1 copyright certificate Educational textbook – №41995 dated January 12, 2024. Title: "Endovascular recanalization in combined atherosclerotic iliac artery disease"
3. Implementation act №9 – June 2025.
4. Patent application for invention submitted (№ 2025/0718.1 dated July 31, 2025), which has passed formal examination and is currently undergoing substantive examination.

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