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Cultivation and cryopreservation of rat stem cells and their interaction with lyophilised acellular matrix

Larysa Fedoniuk
Doctor of Medical Sciences, Head of the Department
I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine
46001, 1 Maidan Voli, Ternopil, Ukraine
https://orcid.org/0000-0003-4910-6888

Alina Dovgalyuk
PhD in Biological Sciences, Associate Professor
I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine
46001, 1 Maidan Voli, Ternopil, Ukraine
https://orcid.org/0000-0003-3976-0245

Olga Furka*
PhD in Biological Sciences, Assistant Professor
I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine
46001, 1 Maidan Voli, Ternopil, Ukraine
https://orcid.org/0000-0002-9917-542X

Ilona Palii
Postgraduate Student
I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine
46001, 1 Maidan Voli, Ternopil, Ukraine
https://orcid.org/0000-0003-0753-8359

Halina Lavrenchuk
Doctor of Biological Sciences, Head of the Laboratory
National Research Center for Radiation Medicine of National Academy of Medical Sciences of Ukraine
04050, 53 Y. Illienko Str., Kyiv, Ukraine
https://orcid.org/0000-0001-7200-712X

Abstract. With the rapid development of regenerative medicine in the 21st century, the study of the therapeutic potential of stem cells in both preclinical research and clinical trials has become particularly relevant. Preclinical studies on animals allow for a detailed understanding of the mechanisms of action of allogzeneic cell preparations, exploring their regenerative activity, pharmacodynamics, and potential side effects. The purpose of the study was to select optimal conditions for obtaining, cultivating, and cryopreserving mesenchymal stem cells from rats and analyse their interaction with the lyophilised acellular matrix. The enzymatic method was applied to obtain primary cell cultures from the umbilical cord, dermis, and muscles of *Rattus norvegicus* fetuses. Cell cultures were cultivated *in vitro*, and cell line proliferation rates were analysed using an inverted microscope. In addition, cryopreservation was performed to store cellular materials. The interaction of mesenchymal stem cells with an acellular matrix and cryopreservation of the obtained cells was at the 4 and 5th passages. It was shown that the optimal nutrient medium for cultivating the obtained lines of mesenchymal stem cells from the umbilical cord and dermis of rat fetuses is DMEM/F12 Advanced. It was established that the method of thawing the cell suspension by 10-fold dilution of dimethyl sulfoxide is more effective than the alternative method of immediate

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*Corresponding author

removal of cryoprotectant by centrifugation. The lyophilised acellular dermal matrix was found to have a cytotoxic effect on all cultured rat cells, while the pericardial matrix showed a positive effect on the growth of the investigated cell lines. Thus, the optimal nutrient medium and conditions for freezing/thawing of rat stem cells were selected, and the effect of lyophilised acellular matrix, planned for therapeutic use, on the obtained cell lines was determined

Keywords: i*n vitro* culture; cryopreservation; umbilical cord; muscles; dermis; lyophilised matrix; pericardium

INTRODUCTION

The last 20 years have witnessed increased interest from biomedical researchers in the use of stem cells (SCs) as an innovative personalised biotechnological method in regenerative medicine. However, before the widespread implementation of cell therapy in clinical practice, it is necessary to comprehensively study the characteristics and mechanisms of action of allogeneic SCs in animal models of various pathologies. Preclinical *in vivo* studies using laboratory animals allow for the collection and analysis of data on therapeutic effects, pharmacodynamics, and potential adverse effects of local or systemic administration of SCs.

Both Ukrainian scientists and researchers from many other developed countries have been exploring the properties of SC for more than 10 years. In particular, H.V. Budash & N.M. Bilko [1], S.K. Ray & S. Mukherjee [2], O. Redko *et al.* [3] described the ability of different types of SCs to differentiate into various cells of the body, which is considered a unique tissue repair system. Other authors revealed the powerful paracrine mechanism of SC secretome in stimulating regeneration and exhibiting immunomodulatory and anti-inflammatory effects [4-6]. In investigating the interaction of allogeneic mesenchymal stem cells (MSCs) with genetically foreign immune cells of recipients, M. Lopes-Pacheco *et al.* [5] and S.S. Chaleshtori *et al.* [6] have shown low immunogenicity of these cells since they weakly express surface human leukocyte antigen (HLA) class I proteins, while HLA class II is only intracellularly expressed and completely absent on the plasma membrane surface. Due to the above characteristics, MSCs evade recognition by alloreactive T lymphocytes. The immunomodulatory effect of MSCs is mainly manifested due to their ability to inhibit the function of various types of pro-inflammatory immune effector cells and activate anti-inflammatory immune responses. According to studies conducted by Chinese scientists, therapeutic effects of MSCs are manifested through their ability to migrate to damaged areas [7-9] and through the production of numerous extracellular vesicles filled with cytokines, growth factors, and microRNAs (miRNAs). It has been shown that molecules secreted by MSCs play an effective role as mediators of regeneration, either by directly activating target cells or by stimulating neighbouring cells to release active repair factors. They are involved in regulating important processes in the recipient's body, such as immune response, maintaining homeostasis, coagulation, inflammation, cancer progression/regression, angiogenesis, and antigen presentation. Thus, substances from MSC-derived extracellular vesicles exhibit their activity in both physiological and pathological conditions [10-12].

Proper and long-term preservation of biological materials remains an important task for medicine and biology. Cryopreservation has been established as a means to provide such capabilities [13]. Scientists have accumulated significant information on preserving the viability of SCs over extended periods of time and the influence of different cryoprotectants on cell survival after deep freezing [14, 15]. Cryopreservation of MSCs can also reduce the constant need for fresh tissues, enable quality control and standardisation of the same cell product at different times when the cellular product is needed.

One important area in regenerative medicine is the development and modification of materials for controlling the viability of SCs, which (re)construct the stromal microenvironment of various human and animal organs and tissues. In addition to the selection of artificial materials capable of biomimetic reproduction of the cellular and tissue microenvironment, the use of decellularised (cell-free) organs and tissues is also relevant for bioengineering purposes [16]. A promising way to treat skin injuries of various etiologies is the use of decellularised pig skin. The main advantages of this biological material are the composition and structure of the pig dermis, which is similar to the human dermis. Skin substitutes have previously been shown to improve wound healing quality and functional treatment outcomes, so they are a useful tool in plastic and reconstructive surgery [16].

This study was conducted as part of the state-funded fundamental research project "Investigation of the Regenerative Potential of Cell Therapy Agents in Acute Respiratory Distress Syndrome" (2021-2023, state registration number 0121U100159) [17].

The purpose of the study was to develop effective techniques for obtaining, culturing, cryopreserving, and thawing rat MSCs and analysing their interaction with dermal and pericardial lyophilised acellular matrices, which were created for the treatment of patients with burn injuries.

MATERIALS AND METHODS

The experiment was conducted in 2021 on the basis of the laboratory of cell cultures of the Ternopil National Medical University of the Ministry of Health of Ukraine. 3 female rats *Rattus norvegicus* were used to obtain primary SC cultures in the late stages of gestation. The animals were kept in standard vivarium conditions (12-hour day/night cycle; t = 20-25°C; humidity 50-55%) with free access to water and food. Euthanasia was performed using thiopental in compliance with bioethical requirements. Umbilical cords, pieces of fetal skin and muscle were taken for experiments. Using a sterile HBSS buffer solution (Gibco) with the addition of 1% penicillin-streptomycin (Sigma), the material was washed from the blood. An enzymatic method using 0.1% collagenase I (Sigma) diluted in DMEM/F12 Advanced nutrient medium (Gibco) was used to dissociate cell mass and produce viable MSCs. Then the material was pipetted and centrifugal for 5 minutes, at a speed of 3000 rpm. The resulting precipitate was resuspended in

a nutrient medium with the addition of 10% fetal bovine serum (FBS)(Gibco) and sown in culture vials, cultured in a CO2 Incubator at a temperature of 37°C and a concentration of CO_2 – 5%. The resulting primary cultures were assigned a zero passage (P_0). Visual assessment of the formation of the monolayer density (confluence) was performed using an inverted Delta Optical NIB-100 microscope.

Cell lines were transported by warm trypsinization (Triple enzymatic solution (Gibco)) when the confluence reached 90-100%. Cell counting was performed using a hemocytometer (Goryaev chamber) using the vital dye Trypan blue.

Cryopreservation of MSCs of the umbilical cord, fetal muscles and skin was performed in passages 4 and 5 after reaching 90% of the confluence. 50% of the cell suspension (2 000 000 cells each) was added to cryoprobes in a DMEM nutrient medium. Then, in 2 steps, a freezing medium was added (30% DMEM/F12 Advanced, 40% FBS, 20% conditioned medium (CS), 10% dimethyl sulfoxide (DMSO) (Sigma)). Further storage of cellular material was conducted at a temperature of -80°C (intermediate stage) and liquid nitrogen (-196°C).

The umbilical cord, dermal, and muscle MSCs at Passages 4 and 5 were used to study the interaction of MSCs with the acellular Matrix. Lyophilised and decellularised pieces of pig dermis and pericardium, 0.5 cm^2 in area, were added to the wells of 6-well culture plates with seeded SCs at the rate of 50,000 cells/ml. The experiment was laid according to the following scheme: control – the cell suspension itself; option 1 – cell suspension + a piece of the dry pericardium; option 2 – cell suspension + a piece of the soaked pericardium in the CS for 1 hour; option 3 – cell suspension + a piece of the dry dermis; option 4 – suspension + a piece of the soaked dermis in the CS for 1 hour. During soaking of the lyophilised material, the CS was changed three times. Each experiment was performed in three repetitions, 6 wells for each point. Microscopic analysis of cell culture growth was performed after 1, 3, and 7 days of the experiment using an inverted microscope. Statistical data analysis was performed using Excel.

All interventions were conducted in compliance with the principles of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" and the law of Ukraine No. 3447-IV "On the protection of animals from ill-treatment" [18, 19].

RESULTS

Obtaining a primary MSC culture. Cell suspensions obtained by fermentation of pieces of the umbilical cord, skin, and muscle of rat fetuses were seeded on culture vials to create conditions for cell adhesion and activation before proliferation. Microscopic analysis of the state of primary cell cultures was performed after 48 hours after sowing. Partial adhesion of polygonal cells was observed in umbilical cord MSC culture (Fig. 1). The cell population density was less than 10%, and there was a slight change in the pH of the medium towards acidic (the acidity indicator phenol red – a mandatory component of the DMEM/F12 Advanced nutrient medium – turned orange). In the SC variants, more cells were attached from the muscle and skin of rat fetuses than in the umbilical cord MSCs variant. The nutrient medium was also slightly acidified. In the primary culture of myogenic MSC cells, the cells were elongated with two diametrically opposite processes, evenly attached to the bottom of the culture plastic, and actively proliferated, covering approximately 70% of the flask area (confluent, accordingly, = 70%). In the case of dermal SCs, the cells were highly flattened, polygonal in shape, forming clones, and reached 50% confluence within the first 48 hours of cultivation (Fig. 2).

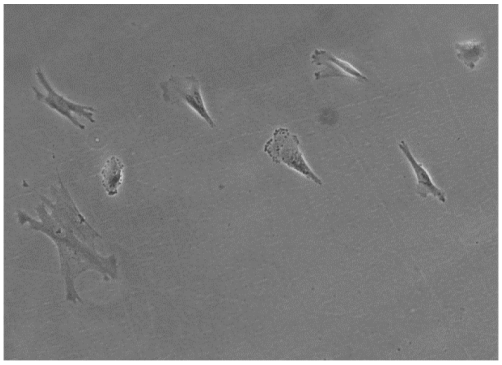

Figure 1. Primary culture of umbilical cord MSCs 2 days after the start of the experiment. Magnification ×100

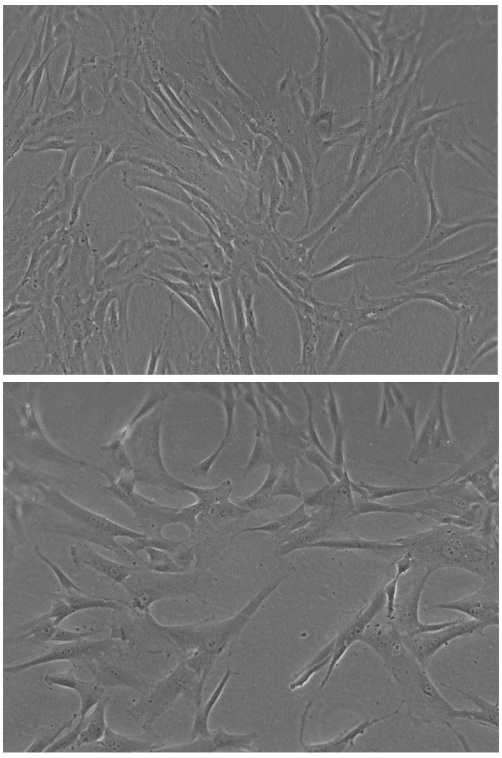

Figure 2. Primary culture of MSCs of muscles and dermis on the third day of the experiment. Magnification ×100

On the third day of cultivation, complete replacement of the nutrient medium was performed in all cell lines while maintaining a 10% FBS concentration. The subsequent analysis of the primary cultures was conducted four days after seeding the material. In the muscle and skin cell lines, the formation of a confluent monolayer (confluence = 100%) was observed, so at this stage, passaging was performed to avoid contact inhibition. In the myogenic cell

variant, occasional myospheroids began to form among the typical fibroblast-like MSCs (Fig. 3).

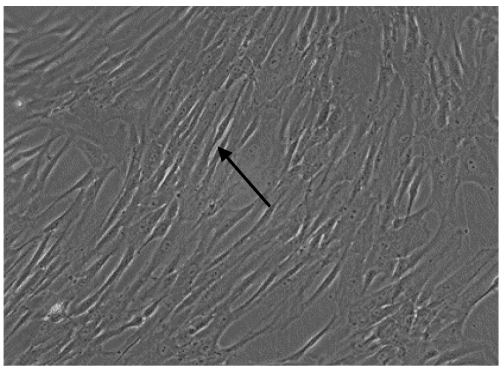

Figure 3. Fusion of myogenic cells with the formation of myospheroids (indicated by an arrow). Magnification ×100

In the umbilical cord MSC culture, after four days of cultivation, the confluence reached approximately 50%, while 100% confluence of umbilical cord cells was achieved only on the 8th day of cultivation. The slow growth rate of this cell population was associated with a lower initial cell seeding density. However, during the first passage, these MSCs increased their proliferation rate to levels comparable to the myogenic cell line. Throughout the first to third passages, the cell lines were cultured in a medium with a reduced FBS concentration of 2%.

Selection of optimal conditions for MSC culture growth. To compare the effects of different nutrient medium compositions on cell growth intensity, two types of culture media, DMEM/F12 and DMEM/F12 Advanced, were used. The experiment was performed on passages 2 and 3. Microscopic analysis of the proliferation rates of cell lines was performed before the formation of 100% confluence. The results of the experiment are presented in Table 1.

Table 1. Dependence of the influence of nutrient media on the intensity of cell proliferation rates, (cell population density, %)

Cell culture	Experimental group	1 days	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days
MSC umbilical cord	DMEM/F12 Advanced	35.0±3	50.0±5	85.0±5	100					
	DMEM/F12	30.0±5	40.0±5	60.0±8	80.0±5	95.0±3	100			
MSC of muscles	DMEM/F12 Advanced	35.0±8	55.0±5	85.0±5	100					
	DMEM/F12	35.0±10	50.0±5	65.0±5	85.0±7	100				
MSC of dermis	DMEM/F12 Advanced	20.0±5	35.0±5	40.0±5	50.0±5	65.0±8	80.0±5	95.0±3	100	
	DMEM/F12	25.0±8	30.0±7	35.0±5	45.0±5	55.0±10	65.0±5	80.0±5	90.0±3	100

Source: compiled by the authors

As evident from the data presented in Table 1, the DMEM/F12 Advanced medium proved to be more effective for the growth of MSC cell populations compared to DMEM/F12. In the umbilical cord MSC line, the first variant reached 100% confluence on the 4th day of cultivation, while the second variant formed a 100% cell monolayer only on the 6th day. In the muscle MSC line, the DMEM/F12 Advanced variant achieved 100% confluence after 4 days of cultivation, while the DMEM/F12 variant reached it on the 5th day. The skin MSC line proliferated significantly slower than the previous two, as indicated by the results. In the DMEM/F12 Advanced variant, 100% confluence was achieved only on the 8th day after the start of the experiment, while in the DMEM/F12 experiment, it occurred on the 9th day.

In addition to examining the influence of nutrient media, the impact of various serum concentrations in the culture medium (DMEM/F12 Advanced + 2%, 5%, 10% FBS, and serum-free medium containing a protein similar to plasma albumin) on the activity of cell lines regarding their growth and proliferation was also investigated. The study was conducted during the 2nd and 3rd passages of the cell lines until 100% confluence was reached.

In the serum-free medium, the peak growth of umbilical cord MSCs was observed on the 7th day, reaching a maximum confluence of 40%. However, the cell line gradually started to die off without reaching 100% monolayer formation. When 2% FBS was added, the cells proliferated smoothly and evenly, achieving a monolayer on the 4th or 5th day of cultivation. Higher serum concentrations in the growth medium promoted accelerated cell proliferation but also induced the formation of spheroids on the 3rd day of cultivation (Fig. 4).

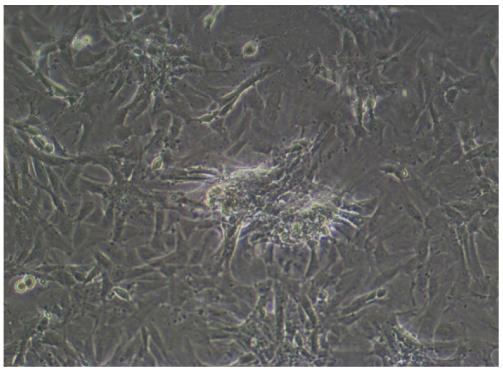

Figure 4. Formation of spheroids in umbilical cord MSC culture under high FBS concentration (10%). Magnification ×100

For muscle MSCs, the optimal serum concentration for culture was also found to be 2% FBS. At 5% and 10% serum concentrations in the growth medium, the proliferation

results were similar to those observed in umbilical cord MSCs at equivalent concentrations. In the serum-free medium, the cell line exhibited unexpected results. On the 11th day of cultivation, it reached 80% confluence, which remained unchanged for 5 days. These findings suggest that the muscle MSC cell line is relatively insensitive to the stimulating effects of serum additives in the growth medium.

The analysis of dermal MSC cell line growth showed that the optimal serum concentration was 5%. At 2% FBS, the cell line proliferated too slowly, with a monolayer forming only on the 9th day. At a concentration of 10%, rapid cell differentiation and acquisition of mature fibroblast morphology were observed.

In the serum-free medium, the cell line paused its proliferation on the 9th or 10th day of cultivation, reaching 60% confluence, and eventually started to die off. Therefore, it can be considered that the optimal FBS concentration is 2% for umbilical cord and muscle MSCs, while it is 5% for dermal MSCs.

Analysis of MSC viability after cryopreservation. One day after thawing samples of the three rat cell lines, it was observed that many cells got attached to the flask surface and acquired the typical morphology characteristic of MSCs. Thawing was performed using two methods: a 10-fold dilution of the cell suspension with freezing medium or immediate removal of the DMSO by centrifugation. In the first thawing method, the cell population density after 24 hours was 15% in all cell lines. At this time, a medium replacement was performed to fully neutralise the effect of the cryoprotectant on the cell populations. In the second thawing variant, the confluence of the umbilical cord MSC line reached approximately 5%, which was the lowest confluence rate. The confluence of the muscle and skin MSC lines was 8% and 10%, respectively. On the 5th day after thawing, the confluence in the case of 10-fold dilution of the cryoprobe content was: 75% for dermal SCs, 55% for skeletal muscle MSCs, and 35% for the umbilical cord MSCs line, and in the variant with centrifuged cells of the same lines – 70%, 60% and 30%, respectively. In the case of 100% diluted cells, a confluence was observed: for skin MSCs cell lines – on the 7th day after defrosting; and for umbilical cord and muscle MSCs – on the 9th day of the experiment. In the variant with centrifuged cells, the dermal MSC population reached a monolayer on the 8th day of cultivation, while the muscle MSCs reached it on the 9th day, and umbilical cord MSCs on the 11th day of the experiment. Thus, the method of thawing cryopreserved cells affects the proliferation rates of MSC cell populations.

Interaction of MSCs with acellular matrix. Analysis of the interaction between extracellular matrix (ECM) and umbilical cord MSCs showed that in the control and pieces of soaked dermis variants, only a small fraction of cells attached on the first day of cultivation. On the third day, cell growth continued in the control wells, and it was observed that cells began to proliferate in the variants with pieces of dry, soaked pericardium and dry dermis. On the 7th day of the experiment, active cell growth was observed in the control, dry pericardium, and soaked pericardium variants. However, in the case of pieces of dry and soaked dermis, the umbilical cord cell line died off (Table 2).

Table 2. Interaction of matrix with MSC cell lines of rats (cell density in wells, %)

Cell line	Experiment variants	Control, %	Dry pericardium, %	Soaked pericardium, %	Dry dermis, %	Soaked dermis, %
MSC umbilical cord	1st day	15 ± 2.7	no attached cells	no attached cells	no attached cells	5 ± 2.8
	3rd day	30 ± 4.2	18 ± 3.0	25 ± 3.0	10 ± 2.0	15 ± 5.1
	7th day	44 ± 3.6	42 ± 3.3	51 ± 3.9	cells are dead	cells are dead
MSC of muscles	1st day	15 ± 2.4	10 ± 2.1	15 ± 3.0	no attached cells	10 ± 4.2
	3rd day	30 ± 4.5	25 ± 3.9	35 ± 5.1	7 ± 2.3	15 ± 2.7
	7th day	51 ± 3.4	47 ± 4.3	58 ± 4.7	cells are dead	cells are dead
MSC of dermis	1st day	23 ± 3.6	15 ± 2.7	20 ± 3.2	5	5 ± 3.2
	3rd day	36 ± 3.7	25 ± 3.8	35 ± 5.6	cells are dead	8 ± 3.6
	7th day	52 ± 6.3	33 ± 3.7	47 ± 3.9	cells are dead	5 ± 2.5

Source: compiled by the authors

In the variant with muscle MSCs, the experiment with pieces of dry and soaked pericardium also showed a relatively favourable effect on the cell line. On the first day of the experiment, cell proliferation began in the control and experimental variants with pericardium and soaked pieces of dermis. By the third day of cultivation, cells proliferated in both the control and all experimental wells. The best result was observed on the 7th day in the variant with soaked pericardium pieces, followed by the control variant and wells with dry pericardium. However, in the case of pieces of dry and soaked dermis, the muscle SC cell line also died off by the end of the experiment (Table 2).

Unlike the previous two MSC cell lines, fetal dermal cells showed a positive result in the variant with soaked pieces of dermis. On the first day after the experiment was initiated, cell proliferation was observed in both the control and all experimental wells. The only difference was that in the presence of acellular dermal matrix, cells were located at the edges of the wells. By the third day, cells in the dry dermis variant had completely died off and detached. The best result was obtained on the 7th day of cultivation in the control and soaked pericardium variants.

DISCUSSION

The obtained results indicate that both types of tested nutrient media, DMEM/F12 and DMEM/F12 Advanced, have a favourable impact on the proliferation of rat MSC cell lines. The difference lies only in the fact that at the same concentration of FBS, cell lines grown in DMEM/F12 medium exhibited slightly slower growth compared to those

cultivated in DMEM/F12 Advanced. This result is obviously due to the special components that are present in the composition of the latter. DMEM/F12 Advanced additionally contains insulin, transferrin, glutathione, ethanolamine, ascorbic acid, and additional protein sources, so it can be used with minimal concentrations of xenogeneic embryonic serum, providing optimal conditions for MSC cultivation. For the cultivation of rat MSCs, an increase in FBS concentration is required when using DMEM/F12 medium. Since the growth rates of dermal SCs were slower compared to umbilical cord and muscle MSCs, it was decided to cultivate dermal cells using DMEM/F12 Advanced supplemented with 5% FBS, while 2% FBS was found to be optimal for myogenic and umbilical cord MSCs.

V.G. Dzhyvak *et al.* [20] outline that blood serum serves as a source of hormones, hormone-like growth factors, and transport proteins for their transfer. In addition, it contains molecules necessary for the formation of the extracellular matrix, facilitating cell adhesion to the culture vessel in *in vitro* cultures [21]. Therefore, for cell cultivation in *in vitro* conditions, the addition of embryonic bovine serum rich in biologically active substances is required [22].

The experiment aimed at determining the optimal FBS concentration in the growth medium revealed that at 2% FBS, cell line proliferation occurred smoothly and gradually. 5% of the serum showed faster cell proliferation and differentiation. This concentration can be used for rapid scaling of MSCs since it accelerates the mitotic division process under the influence of growth factors present in the serum. However, a concentration of 10% FBS proved to be less suitable as, despite achieving full confluence quickly, the investigated SCs started forming specific structures – spheroids (Fig. 4). Similar spheroid formation under the influence of human blood serum was also observed in another study [21]. It is known that elevated concentrations of FBS promote SC differentiation towards adipogenic, osteogenic, or chondrogenic lineages. Therefore, if the goal is to obtain a stable and long-lasting MSC cell line, the use of blood serum should be minimised [23-25]. However, it has been demonstrated that low concentrations of added FBS to the culture medium contribute to the formation of flattened cell monolayers on the culture plastic and prevent spheroid formation [21].

Based on the analysis of scientific sources regarding the cryopreservation of rat MSCs, a freezing medium was selected consisting of 30% DMEM/F12 Advanced, 40% FBS, 20% CS, 10% DMSO, and containing minimal amounts of cryoprotectant [13, 14]. From a detailed review by M. Awan *et al.* [15], it is known that although DMSO is a widely used and effective cryoprotectant, it exhibits toxic effects on cultured cells during prolonged exposure in the thawed state. However, a study by A. Mitchell *et al.* [26] demonstrated that a high concentration of FBS in the freezing medium mitigates the harmful effects of DMSO. CS also possesses protective properties for MSCs as it contains exosomes with SC secretome in addition to the products of cell metabolism [27]. Since the freezing medium was mixed with the cell suspension at a 1:1 ratio, the final concentration of cryoprotectant decreased to 5%, and its potential toxic effects were minimised by the higher concentration of FBS. As a result, the survival rate of rat MSCs in this study exceeded 85-90% after thawing. The method of thawing cell suspensions by 10-fold dilution of the cryoprotectant was found to be more effective than the alternative method of immediate removal of DMSO by centrifugation. Consequently, centrifugation of MSCs in the presence of DMSO proved to be more detrimental to the cells than their cultivation for 24 hours in the presence of a highly diluted cryoprotectant solution.

The analysis of the interaction between xenogeneic lyophilised acellular matrix and umbilical cord, muscle, and dermal MSCs revealed that for all cell lines, pericardium exhibited the most favourable interaction, particularly when it was rinsed three times in the growth medium beforehand. However, in the variants with the addition of decellularised dermal tissue fragments to the culture medium, the survival of rat SCs was observed to decline. This indicated a toxic effect of this material on the cell cultures. Studies by D.D. Mathew *et al.* [28] and R. Nassiri Mansour *et al.* [29] have shown that for the creation of bioengineered constructs for tissue and organ regeneration, autologous, allogeneic, or xenogeneic MSCs can be successfully cultured in acellular matrices. Such acellular matrices typically provide the necessary support for cell adhesion, proliferation, phenotypic differentiation of SCs, and offer favourable biochemical and biophysical conditions for tissue modelling and formation of neotissues. Therefore, based on a careful analysis of the method of obtaining the used acellular dermal matrix, it was presumed that its cytotoxic effect arises from the presence of detergents [16]. The conducted experiment revealed the necessity of modifying the protocol for obtaining acellular dermal matrix before its application in burn care practice.

CONCLUSIONS

Thus, this study demonstrates that the enzymatic method using 0.1% collagenase I is suitable for obtaining primary cultures of MSCs from umbilical cords, muscles, and dermal tissue of rat fetuses. For the cultivation of the obtained umbilical cord and dermal MSC lines, DMEM/F12 Advanced medium is optimal due to the presence of effective additives that are absent in DMEM/F12 medium. Since the culture of rat myogenic cells exhibited higher proliferative potential compared to other investigated MSC lines, it can also be cultivated in DMEM/F12 medium. It was also determined that a 2% concentration of FBS is optimal for efficient proliferation of umbilical cord and muscle MSCs, while a 5% FBS concentration is optimal for the dermal MSC line. In some cases, muscle MSCs can be cultured in a growth medium without the addition of serum.

Thawing the cell suspension by 10-fold dilution of DMSO was found to be a more effective method, as the alternative method of immediate removal of the cryoprotectant by centrifugation slowed down the growth of rat MSCs. Thus, centrifugation of MSCs in the presence of DMSO was more harmful to the cells than their cultivation for 24 hours in the presence of a highly diluted cryoprotectant solution. The analysed lyophilised acellular matrix in the form of fragments of dry dermal tissue exhibited a toxic effect on all investigated MSC cell lines. Conversely, dry and rehydrated lyophilised pericardium, when cultured in a growth medium, not only had no harmful effect but sometimes even stimulated the growth of SCs. Therefore,

lyophilised pericardium is recommended for the treatment of skin defects.

For the clinical use of decellularised porcine dermal matrix, it is necessary to improve the methodology of obtaining this material by increasing the number of washes after detergent treatment to reduce or eliminate its cytotoxic effects.

The next stage of comprehensive research is planned to investigate the mechanisms of the therapeutic effects of systemically administered allogeneic MSCs in rats with experimentally induced acute respiratory distress syndrome.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Особливості культивування та кріоконсервування стовбурових клітин щурів та їх взаємодія із ліофілізованим ацелюлярним матриксом

Лариса Ярославівна Федонюк
Доктор медичних наук, завідувач кафедри
Тернопільський національний медичний університет імені І. Я. Горбачевського МОЗ України
46001, майдан Волі, 1, м. Тернопіль, Україна
https://orcid.org/0000-0003-4910-6888

Аліна Іванівна Довгалюк
Кандидат біологічних наук, доцент
Тернопільський національний медичний університет імені І. Я. Горбачевського МОЗ України
46001, майдан Волі, 1, м. Тернопіль, Україна
https://orcid.org/0000-0003-3976-0245

Ольга Богданівна Фурка
Кандидат біологічних наук, асистент
Тернопільський національний медичний університет імені І. Я. Горбачевського МОЗ України
46001, майдан Волі, 1, м. Тернопіль, Україна
https://orcid.org/0000-0002-9917-542X

Ілона Русланівна Палій
Аспірант
Тернопільський національний медичний університет імені І. Я. Горбачевського МОЗ України
46001, майдан Волі, 1, м. Тернопіль, Україна
https://orcid.org/0000-0003-0753-8359

Галина Йосипівна Лавренчук
Доктор біологічних наук, завідувач лабораторії
ДУ Національний науковий центр радіаційної медицини НАМН України
04050, вул. Ю. Іллєнка, 53, м. Київ, Україна
https://orcid.org/0000-0001-7200-712X

Анотація. Через стрімкий розвиток регенеративної медицини у XXI столітті особливої актуальності набувають вивчення терапевтичного потенціалу стовбурових клітин як у доклінічних дослідженнях, так і в клінічних трайлах. Доклінічні дослідження на тваринах дають змогу в деталях з'ясувати механізми дії алогенних клітинних препаратів, вивчити їхню регенеративну активність, фармакодинаміку та можливі побічні ефекти. Метою дослідження був підбір оптимальних умов для отримання, вирощування та кріозберігання мезенхімальних стовбурових клітин щурів та аналіз їхньої взаємодії з ліофілізованим ацелюлярним матриксом. У ході дослідження було застосовано ферментативний метод для отримання первинних культур клітин з пуповини, дерми та м'язів плодів *Rattus norvegicus*. Культури клітин були культивовані *in vitro*, а темпи проліферації клітинних ліній були аналізовані за допомогою інвертованого мікроскопа. Крім того, здійснювалося кріоконсервування для зберігання клітинних матеріалів. Взаємодію мезенхімальних стовбурових клітин із ацелюлярним матриксом та кріоконсервацію отриманих клітин проводили на 4 та 5 пасажах. Показано, що для вирощування отриманих ліній мезенхімальних стовбурових клітин з пуповини та дерми плодів щурів оптимальним є поживне середовище DMEM/F12 Advanced. Встановлено, що спосіб розморожування клітинної суспензії шляхом 10-кратного розведення диметилсульфоксиду є ефективнішим за альтернативний спосіб негайного усунення кріопротектора за допомогою центрифугування. З'ясовано, що ліофілізований ацелюлярний дермальний матрикс цитотоксично впливає на усі культивовані щурячі клітини, в той час, як перикардіальний матрикс проявляв позитивний ефект на ріст досліджуваних клітинних ліній. Таким чином, підібрано оптимальне живильне середовище та умови для заморожування/розморожування стовбурових клітин щурів, а також визначено вплив на отримані клітинні лінії ліофілізованого ацелюлярного матриксу, що планувався для терапевтичного використання

Ключові слова: культура *in vitro*; кріоконсервація; пуповина; м'язи; дерма; ліофілізований матрикс; перикард