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Effect of Cryopreservation on Different Passages of Porcine Dorsal Root Ganglion Cell Culture

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Effect of Cryopreservation on Different Passages of Porcine Dorsal Root Ganglion Cell Culture

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Abstract
The cell culture obtained from dorsal root ganglia (DRG) is a valuable model used in biology or medical research. However, the effect of cryopreservation on the properties of DRG-derived cell culture of different passages remains unclear up to date. The objective of the study is to assess the effect of cryopreservation with various concentrations of cryoprotectant dimethyl sulfoxide (DMSO) on the viability and morphological features of porcine neonatal DRG cell culture of different passages. Cell suspension was obtained from DRG of neonatal piglets and cultured in an α-MEM nutritional medium supplemented with 10% fetal calf serum (0, 1st, and 2nd passages). Cells of different passages were cryopreserved at a cooling rate of 0.5°C/min to −20°C (step 1) and 1°C/min to −80°C (step 2) followed by immersion into liquid nitrogen. Cryoprotective solutions based on α-MEM nutrient medium and 25% fetal calf serum (FCS) containing 5%, 7.5%, and 10% DMSO were used. It was established that the primary culture (passage 0) consisted of four cell morphological types: large rounded cell bodies of sensory neurons (SN) and three types of non-neuronal cells, namely, polygonal cells with pronounced elongated processes (type 1), spindle-shaped cells (type 2), and multipolar flattened fibroblastoid cells (type 3). As the number of passages increases, an elimination of SN from the culture, a decrease in the relative number of 1st and 2nd cell types, and expansion of 3rd cell type were observed. DRG cell culture had sufficiently high resistance to cryopreservation as cell viability was in the range from 83% to 90% using different concentrations of DMSO. The cells of passage 1 were more resistant to cryopreservation in comparison with primary culture cells (passage 0). The best result was achieved by freezing the culture of passage 1 in the cryoprotective medium with 7.5% DMSO, where 90.6% of viable cells were observed after thawing.

Keywords: Cell culture; Dorsal root ganglia; Satellite glial cells; Dimethyl sulfoxide; Cryopreservation.

1. INTRODUCTION

The cell culture obtained from dorsal root ganglia (DRG) of embryonic and neonatal animals is widely used in neurobiology because it contains neurons, glial cells, and neural stem cells and has good growth properties [1-4].

Cryopreservation is a modern method that enables storing of cell culture samples for a long time, certify them, and use as necessary. Currently, methods for cryopreservation of isolated sensory neurons (SN) of dogs and rats have been described [5, 6], however, there is no information on the sensitivity of other DRG-derived cells to freezing. The primary culture obtained by DRG enzymatic processing and cell disaggregation contains almost all conventional cell types. Based on this, it is a good model to study the sensitivity of different DRG cell types to cryopreservation.

The simplest and most widely used method for obtaining a culture enriched with stem/progenitor cells is subculturing, in which highly differentiated cells are eliminated with passages [7, 8]. In addition, subculturing is an important stage to increase the number of cells required for use in the framework of the assigned tasks. However, till date the effect of cryopreservation on the composition and properties of DRG cell culture of different passages have not been practically studied.

The objective of study is to assess the effect of cryopreservation in the presence of various concentrations of cryoprotectant dimethyl sulfoxide (DMSO) on the viability and morphological features of porcine neonatal DRG cell culture of different passages.

2. METHOD(S)

Cell suspension was obtained from DRG of 1-day-old piglets by the method of de Luca et al. [9]. The harvested cells were seeded at a concentration of 5×10^5 cells/ml on poly-D-lysine-coated Petri dishes (Orange Scientific, Belgium). The cells were cultivated

in an α-MEM nutritional medium (Biowest, France) with 10% fetal calf serum (FCS, BioSera, France) and antibiotics at 37°C in 5% CO_2 atmosphere. For sub-culture seeding, 70% cell monolayer was detached from the surface with a solution containing 0.2 g/L EDTA and 0.25% trypsin (Biowest, France); cells were diluted with fresh nutrient medium in a ratio of 1:15 and further cultured under abovementioned conditions.

For cryopreservation, α-MEM and 25% FCS solutions prepared on the basis of the nutrient medium and containing DMSO (AppliChem, Germany) with 5%, 7.5%, and 10% concentrations were used. Cells of primary culture (passage 0) and passage 1 were diluted with an equal volume of a medium precooled to 10°C and containing a double concentration of DMSO. The cells were incubated with a cryoprotectant for 10 minutes, after which they were frozen at a cooling rate of 0.5°C/min to −20°C (step 1) and 1°C/min to −80°C (step 2) followed by immersion into liquid nitrogen. The cells were thawed in a water bath at a temperature of 37°C, washed in an α-MEM nutrient medium by centrifugation, and cultivated under the above conditions. The cells of the intact culture of the corresponding passage after detachment from the surface serve as a control.

In viability assay, the cells were stained with 0.4% trypan blue solution in a ratio of 1:1. The viability was determined as the ratio of a number of unstained cells to a total cell number and expressed as a percentage. For morphological analysis, a cell monolayer was fixed in 4% paraformaldehyde (Sigma, USA) for 15 minutes, then washed with phosphate-buffered saline (PBS; pH = 7.4) and stained with hematoxylin and eosin.

The experimental data are presented as mean values ± standard deviation (m ± sd). The type of distribution was determined using the Shapiro–Wilk W-criterion, and the significance of differences between data groups was calculated using ANOVA. The differences were considered statistically significant at $p < 0.05$.

3. RESULTS

The composition of the primary DRG cell culture (passage 0) was heterogeneous (Figure 1a). The clusters of large rounded cells representing the bodies of SN [10] and three types of non-neuronal cells—polygonal cells with pronounced flattened processes (type 1), spindle-shaped cells (type 2), and multipolar flattened fibroblastoid cells (type 3) were observed. The cells of 1st and 2nd types prevailed in the primary culture, as their numbers were 49.5 ± 2.4% and 41.7 ± 1.5%, respectively (Figure 2). The numbers of SN and the 3rd type cells were 3.5 ± 0.6 and 5.3 ± 1.9%, respectively.

Subculturing led to elimination of SN, decrease in the number of spindle-shaped cells (type 2), and expansion of the 3rd type cells (Figure 1b and c). Therefore, 50.8 ± 1.9% of 3rd type cells, 37.0 ± 2.2% of 2nd type cells, and 12.3 ± 1.5% of 1st type cells were observed at passage 1; and 66.0 ± 1.8% of 3rd type cells, 29.0 ± 1.8% of 2nd type cells, and 5.0 ± 0.8% of 1st type cells were observed at passage 2 (Figure 2).

It was established (Figure 3) that cryopreservation of primary culture (passage 0) in the presence of 5% and 10% DMSO reduced cell viability from 92% to 83% ($p < 0.05$) in comparison with the intact control, and in the presence of 7.5% DMSO to 87.7% ($p < 0.05$). The cells of passage 1 were more resistant to cryopreservation. When the subculture was frozen in the presence of 5% DMSO, cell viability decreased to 84.9% ($p < 0.05$), and in the presence of 7.5% and 10% DMSO it did not significantly differ from that of the control.

It is known that the process of cryopreservation does not equally influence the survival of various types of cells in a heterogeneous culture. In this regard, we analyzed the ratio of cell types in a culture frozen at various passages.

Cryopreservation at passage 0 changed the morphological composition of the culture toward an increase in the 1st type cells (Figure 4). The closest to control by the ratio of cell types was a culture frozen in the presence of 10% DMSO.

Figure 1: Neonatal porcine DRG cell culture on the 3rd day after seeding: a—passage 0, b—passage 1, c—passage 2. A white arrow is a cluster of SN bodies, 1—cells of type 1, 2—cells of type 2, 3—cells of type 3.

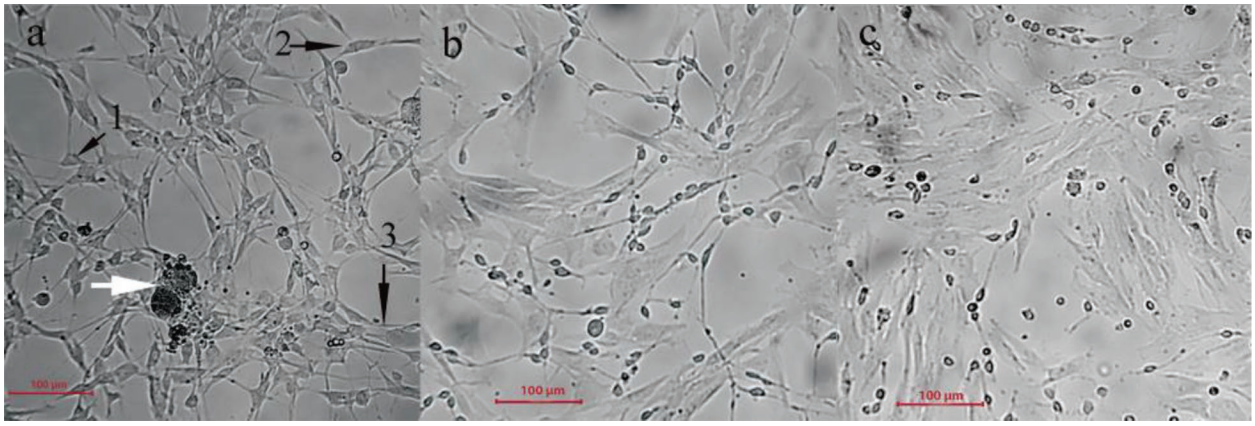
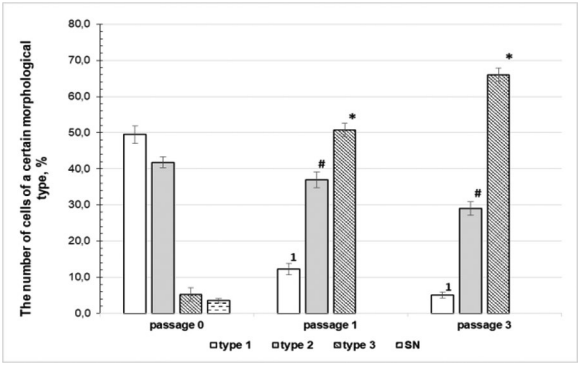
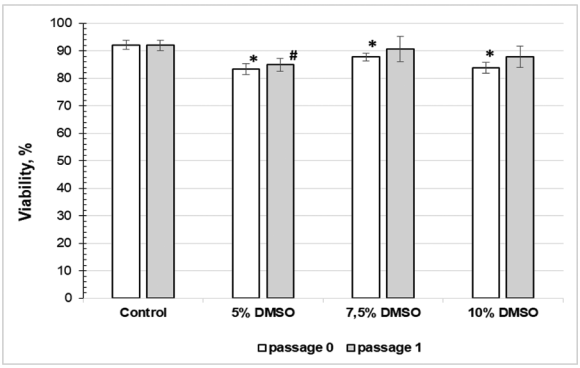


Figure 2. Number of the cells of various morphological types in neonatal porcine DRG cell culture of different passages.



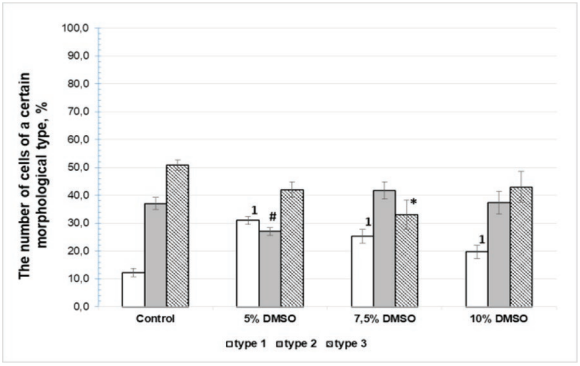
1—type 1 in relation to passage 0, at $p < 0.05$
#—type 2 in relation to passage 0, at $p < 0.05$
*—type 3 in relation to passage 0, at $p < 0.05$.

Figure 3. Viability of the cells of different passages after cryopreservation.



*—the difference is statistically significant in relation to the control (passage 0), at $p < 0.05$
#—the difference is statistically significant in relation to the control (passage 1), at $p < 0.05$

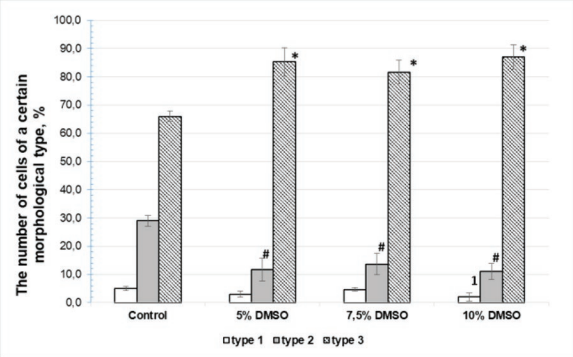
Figure 4: Number of cells of various morphological types in DRG cell culture cryopreserved at passage 0.



1—type 1 in relation to control, at $p < 0.05$
#—type 2 in relation to control, at $p < 0.05$
*—type 3 in relation to control, at $p < 0.05$.

 https://doi.org/10.18639/RABM.2019.941755

Figure 5: Number of cells of various morphological types in DRG cell culture cryopreserved at passage 1.



1—type 1 in relation to control, at $p < 0.05$
#—type 2 in relation to control, at $p < 0.05$
*—type 3 in relation to control, at $p < 0.05$.

During cryopreservation at passage 1, it was revealed that, as compared with passage 0, the sensitivity of the 2nd type cells to cryopreservation increased (Figure 5). The number of these cells after thawing was from 11% to 14% whereas in the intact control it was 29%. The 3rd type cells were most resistant to freezing because their number ranged from 81% to 87% depending on DMSO concentration. In the intact culture of the corresponding passage, the number of cells of this type was 66%.

4. DISCUSSION

In the DRG, the somas of neurons are surrounded by satellite glial cells, and their axons are myelinated by Schwann cells. A ganglion is surrounded by a connective tissue capsule, and between nerve fibers there is an endoneurium with blood vessels passing through it. Thus, primary DRG cell culture may contain neurons, satellite glial cells, Schwann cells, endotheliocytes, and fibroblasts. Considering this fact, it was topical to analyze the viability and ratio of cytomorphological types after cryopreservation of DRG cell culture at various passages.

In this study, we showed that DRG cell culture of neonatal piglets has sufficiently high resistance to cryopreservation regardless of passage. Using slow cooling rates and DMSO concentrations from 5% to 10%, cell viability ranged from 83% to 90%.

The cells of passage 1 were more resistant to cryopreservation compared with those of passage 0. In this, our data agree with the results of other authors [11], in which a change in the sensitivity of fibroblasts to cryopreservation depending on a number of passages was established. Probably, an increase in resistance is associated with elimination at the 1st passage of cells bearing lethal damages due to processing DRG tissue. The best result was achieved by freezing the cells of passage 1 using slow cooling at rates of 0.5°C/min to −20°C in stage 1 and 1°C/min to −80°C in stage 2 followed by immersion in liquid nitrogen in a cryoprotective medium based on 7.5% DMSO. It enabled to preserve 90.6% of viable cells after thawing.

The change in the ratio of morphological cell types in the culture occurred depending on both a passage number and cryopreservation. The chosen cultivation conditions (the absence of Nerve Growth Factor [NGF] and the presence of 10% FCS) contributed to the maintenance of satellite glial cells but not neurons [12-15]. In this regard, the result of the elimination of SN from the culture seems logical.

The morphological plasticity of satellite glial cells during cultivation is well known. In vitro, they can acquire a different shape: polygonal, spindle-shaped, fibroblastoid, and rounded [12-15]. However, regardless of morphology they have phenotypic features of neuroglia including the expression of Glial fibrillary acidic protein (GFAP) and glutamine synthetase [12, 15]. Thus, three cytomorphological types observed in neonatal porcine DRG culture are most likely satellite glial cells. It is interesting that an expansion of 3rd type fibroblastoid cells, which initially represented a small percentage of the total quantity, was observed in passages. Moreover, they were found to be the most resistant to cryopreservation. Further studies are required to assess phenotypic characteristics of the cells of various morphological types observed in DRG culture of different passages.

5. CONCLUSION

1. Quantity of passages influences a morphological type of cell that prevails in neonatal porcine DRG cell culture. As the number of passages increases, an elimination of SN, a decrease of the 1st type cells (polygonal cells with pronounced flattened processes) and 2nd type cells (spindle-shaped cells), and expansion of 3rd type cells (fibroblastoid cells) are observed.

2. Cryopreservation reduces the viability of DRG cell culture; at this, the 1st passage cells are more resistant to cryopreservation in comparison with primary culture cells (passage 0).
3. The best result was achieved by freezing the culture of passage 1 in a cryoprotective medium based on 7.5% DMSO, where 90.6% of viable cells were observed after thawing. At this, the culture was mainly represented by the 3rd type cells (>80%).

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Author Contributions
All authors contributed equally to this study.

Conflict of Interest
None.

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