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CRYOPRESERVATION OF NEURAL CREST-DERIVED CELLS

Neural crest-derived cells are formed from neuroectoderm at early stages of embryogenesis. As a result of the epitheliomesenchymal transition, they migrate to peripheral structures and differentiate into neurons and glia of the peripheral nervous system, skin melanocytes, dental pulp cells, neuroendocrine cells, cartilage and bones of the skull and several other phenotypes. Neural crest derivatives have great prospects for application in regenerative medicine. Cryopreservation is widely used for long-term storage of biological material, enabling its application in clinical practice. Here, we have avalyzed the approaches to cryopreservation of neural crest-derived cells procured from different sources. The in vitro and in vivo studies demonstrate the effectiveness of the developed protocols for cryopreservation of various neural crest-derived cells, which opens the way for establishing cryobanks and expanding the use of these cells in clinical practice.

Key words: *cryopreservation, neural crest, 2D- culture, 3D-culture, dorsal root ganglion cells, dental pulp cells, melanocytes, Schwann cells.*

The neural crest (NC) is a transient structure that forms from the neuroectoderm in the early stages of embryogenesis. As a result of epithelial-mesenchymal transition, the neural crest-derived cells (NCDCs) migrate to peripheral structures and differentiate into cell types such as neurons and glia of the peripheral nervous system, melanocytes, dental pulp cells, neuroendocrine cells, cartilage and bone of the skull, and other phenotypes [40, 46, 68].

To date, the migration of NC cells has been found to occur in waves [2, 37, 40]. It is known that there is a certain correlation between the initial localisation and migration periods of NCDCs and their potential for differentiation [2, 17]. NC-derived cells are formed along the entire length of the neural tube: from the diencephalon to sacral compartments below somite level 28. Migrating from different parts of the neural tube, NC cells are involved in formation of different structures, and so there are several sections in the NC. D. Raible *et al.* [61, 62] and J.H. Dawes *et al.* [17] have found that early migratory subpopulations of cells differentiate mainly into sensory and sympathetic ganglia, Schwann cells, and pigment cells. From the next migratory pool of cells, the glial and pigment cells are formed. Cells of the late stages of migration differentiate mainly into melanocytes [17].

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During migration to 'destination', a gradual determination and specialization of NCDCs occur. Cranial NC cells migrate to the pharyngeal arches and face, forming bones and cartilage of face and neck, pigment cells and cranial nerves [25]. The vagal (at somite level 1—7) and sacral (below somite 28) NC sections form the parasympathetic nerves of the intestine. The cells of the NC cardiac section migrate at somite level 1—3 and participate in formation of septa between the aorta and pulmonary trunk, as well as between the right and left ventricles of the heart. NC-derived cells at somite level 6–24 form sympathetic neurons and adrenal medulla (somites 18—24).

Multipotent neural creast stem cells (NCSCs) are present not only in the NC of embryos, but also in various fetal tissues and even in the adult body [41, 45, 49]. NCSCs have been identified in the hair follicle of adult mammals [8, 45, 66]. There is evidence that postnatal NCSCs have a low proliferation rate [8, 74] and differentiate into a narrower range of neuronal subtypes [38, 48]. However, they could potentially be an available source of stem cells for regenerative medicine [10, 68, 72].

Cryopreservation is widely used for long-term storage of biological material, including its further use in clinical practice [47]. Various cryopreservation protocols have been developed, which mainly differ in freeze-thawing rate, cryoprotectants used, the techniques of cell saturation with cryoprotective medium, cell density in the sample, and methods of cryoprotectant removal. Varying these parameters enables to create a cryopreservation protocol that is relevant for a particular cell type. Since NCDCs are a heterogeneous population of cells characterised by high plasticity, it is important to understand the changes in their cryosensitivity during differentiation to develop an optimal cryopreservation regimen.

Given the above, we consider here whether the cryopreservation of NCDCs depends on their origin from certain regions of the neural tube (cranial, vagal, trunk and sacral ones).

CRYOPRESERVATION OF DORSAL ROOT GANGLION CELLS (MIGRATION FROM THE TRUNK AND SACRAL REGIONS OF THE NEURAL TUBE)

Dorsal root ganglia are anatomically composed of several cell types. They contain the bodies of sensitive unmyelinated and myelinated neurons that transmit sensory information from the periphery to the CNS [63]. Satellite glial cells within the dorsal root ganglia are specialized cells that surround the neuronal bodies and provide regulatory and metabolic support. The dorsal root ganglia also contain endoneurial connective tissue cells and Schwann cells, which are involved in formation of myelin sheath of axons.

The cytotoxic effect of DMSO on various cells, including cells of neural origin, has been reported many times [13, 30, 60, 75, 77]. Even in low concentrations, DMSO can have a damaging effect. For example, a 12-hour *in vitro* incubation of neonatal rat neurons with DMSO at a concentration of 1 % or higher caused a sharp decrease in neurons' viability, a pronounced disorder of their morphology, and a reduction in NeuN expression [77]. J.L. Hanslick *et al.* [30] showed that low concentrations of DMSO (0.5 and 1 %) resulted in a death of about 50 % of rat hippocampal neurons. In astrocytes, a 1 % DMSO after a 24-hour incubation caused mitochondrial swelling, disorder of membrane potential, decreased levels of cytochrome and glial glutamate transporter (GLT-1) expression, and at a concentration of 5 %, it led to apoptosis [75]. DMSO was also found to affect the DNA methylation and histone modification via regulating the expression of epigenetic modification enzymes [13].

The results of determining the optimal cooling rate for sensory neurons showed a certain dependence of cell survival on membrane fluidity and cell size [42]. It has been found that during differentiation of sensory neurons, the fluctuations in their membrane fluidity occur. It is during the time interval characterised by the maximum membrane fluidity that the minimum intracellular ice formation is observed at a cooling rate of 1 °C / min and 88 % of cells capable of adhesion are preserved after freeze-thawing. As reported by K. Yamatoya *et al.* [73], the efficiency of preservation of highly differentiated neural cells after freezing was much lower compared to low-differentiated ones.

In addition to membrane fluidity, cell size is considered to be another factor that affects cell sensitivity, including NCDCs, to freezing. It is generally accepted that cryodamage is directly related to changes in aggregate state and phase transitions in a cell and extracellular environ-

ment. When a temperature below zero is reached, an extracellular water crystallizes first, followed by intracellular crystallisation [23]. The intracellular ice formation is one of the major causes of cell death. It has been shown that, under the same experimental conditions, the cell volume, which is related to the amount of intracellular water is crucial in frequency of intracellular crystallisation [59]. Herewith, cells with a larger diameter have an increased probability of intracellular ice formation than those with a smaller diameter [59]. This statement is consistent with the data of R. Li *et al.* [42] on fluctuations in diameter of sensory neurons during their differentiation: a high cryoresistance was observed in immature cells, which were the smallest among those studied.

It was found that after cryopreservation of dog dorsal root ganglion neurons at a cooling rate of 1°C / min with 10 % DMSO and 31 % fetal bovine serum (FBS), their viability and total number significantly decreased [64]. In addition, a decrease in neuronal soma size and inhibition of the ability to form processes *in vitro* were observed.

Cryopreservation at a rate of 0.5—1 °C / min with 5, 7.5 and 10 % DMSO and 25 % FBS preserved an adhesive capacity of dorsal root ganglion cells of newborn piglets, but reduced proliferative activity during cultivation [4]. The optimal concentration of DMSO for maintaining cell viability at 87 % and the high proliferative activity (85 % of the intact control' monolayer relative area) was 7.5 %. Under these conditions, the number of satellite glial cells in the culture was about 95 % after thawing.

It has been shown that encapsulation into collagen hydrogel affects the cryopreservation outcome for chicken embryo dorsal root ganglia [16]. Cryopreservation of untreated specimens with 10 % DMSO and 30 % FBS at –80 °C followed by immersion into liquid nitrogen resulted in a decreased viability of cells within the ganglion to an average of 46 %. However, if the dorsal root ganglia were cultured on the surface covered with a collagen hydrogel, the viability of their cells after freezing was about 84 %. The ganglia retained intact morphological features, had high expression of Schwann cell biomarkers and a lower percentage of apoptosis.

CRYOPRESERVATION OF PERIPHERAL NERVE AND SCHWANN CELLS

Slow freezing as an optimal approach to long-term storage of peripheral nerves has been suggested in several reserches [21, 33, 76, 78, 80]. Based on the analysis of the reported data, to prevent intracellular ice formation in nerve fragments and maintain their structural integrity and Schwann cell population, a cooling rate within 1—4 °C / min range is recommended. In addition, a rapid thawing of cryopreserved nerve can enhance the Schwann cells survival [80].

DMSO and glycerol are the most commonly used cryoprotective agents for nerve cryopreservation [33, 70, 76, 78, 80]. A. Zalewski *et al.* [76] have compared the effects of glycerol, DMSO, and a mixture of DMSO and formamide in a 2:1 ratio. The highest survival of frozen-thawed nerves after allografting was demonstrated with a 20 % DMSO / formamide mixture (saturation time of 20 min, a cooling rate of 1—1.5 °C / min). The allografts had well-preserved histological elements, including myelinated and non-myelinated axons, Schwann cells, endoneurium, perineurium, and blood vessels.

S.A. González Porto *et al.* [28] have cryopreserved the rat peripheral nerve fragments in 199 medium with 10 % DMSO alone or in combination with 4 % human albumin (in the latter case, during thawing, the samples were successively washed with saline supplemented with 20 % human albumin). The cooling rate was the same for both groups and made 1 °C / min down to –40 °C, then 5 °C/min down to –140 °C. Histological analysis of the nerve after cryopreservation revealed the preservation of its structural integrity in both groups. However, the Schwann cell viability was significantly higher in nerve fragments cryopreserved with albumin addition.

The method of cryopreservation for primary Schwann cell cultures derived from human peripheral nerve was described by N. Andersen *et al.* [5, 6]. The authors used either the cryoprotective medium consisting of 90 % FBS and 10 % DMSO [5] or a commercial cryopreservation medium 'Recovery™ Cell Culture Freezing Medium' (Thermo-Fisher, USA) [6] and a freezing container 'Mr. Frosty™ Freezing Container' (Thermo-Fisher), which provided

1 °C / min freezing rate in a freezer at –80 °C. The viability rate of Schwann cells after thawing was more than 90 %. T.E. Trumble *et al.* [70] also found that 10 % DMSO was the optimal concentration for cryopreservation of primary Schwann cell culture.

The primary Schwann cell culture can be procured not only from fresh but cryopreserved nerve as well. In the work of P.W. Mason *et al.* [44], the peripheral nerve fragments were placed in a cryoprotective medium with 25 % DMSO and 50 % FBS in a freezer at –80 °C for 24 hrs and then stored in liquid nitrogen until use. Schwann cells derived from cryopreserved nerve were similar to fresh ones by phenotypic features and response to specific mitogens.

CRYOPRESERVATION OF SKIN NEURAL CREST-DERIVED CELLS (MIGRATION FROM THE CRANIAL, VAGAL, TRUNK AND SACRAL PARTS OF THE NEURAL TUBE)

Mammalian skin-derived multipotent NCDCs have the potential to differentiate into neurons, glia, smooth muscle cells and adipocytes [69] and express the NC markers *Slug, Snail, Twist, Pax3* and *Sox9* [22]. The dermal papilla is believed to be an endogenous niche for NCDCs in the facial skin [19]. Skin melanocytes are neural crest derivatives as well [14].

At present, the hair follicle-derived NCDCs are recognized as a promising and available source of multipotent stem cells [24].

The cryopreservation of the adult human hair follicle cells in a cryoprotective medium based on 90 % FBS and 10 % DMSO enabled to achieve 82.2 % of viable cells, showing the preservation of both neuronal and glial differentiation potential [24].

W. Cao *et al.* [12] have compared the cryoprotective media with 10 % glycerol or DMSO for cryopreservation of the whole mouse vibrissal follicles. Compared to glycerol, the DMSO-based medium preserved stem cell populations more efficiently and promoted the functional recovery of follicular cell growth after subcutaneous transplantation.

S. Kajiura *et al.* [35, 36] have developed an optimal protocol for cryopreservation of the whole mouse hair follicles and demonstrated the advantage of slow freezing compared to vitrification. Cryopreservation of hair follicles in a commercial cryoprotective medium 'TC-Protector medium' (DS Pharma Biomedical Co., Japan) with a cooling rate, provided by keeping the cryotube in the freezer at –80°C for a day allowed to obtain about 90 % of post-thawed follicles showing cell migration. Cells derived from frozen-thawed follicles retained their ability to proliferate and expressed the stem cell markers nestin, Sox2, and SSEA-1.

Cells isolated from the dermal papilla of a newborn rabbit hair follicle (vibrissa) were cryopreserved by O. Novikova *et al.* [50]. Cryopreservation of cells at a cooling rate of 1 °C / min down to -80°C and subsequent immersion in liquid nitrogen in DMEM-based cryoprotective medium supplemented with 5 % bovine serum albumin and DMSO at 5—10 % concentrations enabled to maintain cell viability at 90—93 % immediately after thawing. However, the cells occurred to be quite sensitive to a toxic effect of DMSO at a concentration of 10 %, as manifested by an increased number of cell mitotic pathologies, a redcued mitotic index in the first passage, or a complete cessation of cell proliferation in the second one [52].

A high sensitivity of melanocytes to damaging factors of cryopreservation has been established [1, 15]. C.C. Comton *et al.* [15] have described a 77 % decrease in survival of primary culture of melanocytes procured from human skin after cryopreservation with 10 % glycerol. Further, under repeated subcultivation, alternating with freezing, melanocytes were not preserved at all.

The preservation of morphology and functions of human epidermal melanocytes and their proliferation *in vitro* after freezing was established by X. Cai *et al.* [11]. In this case, freezing was performed under a high-voltage electrostatic field (HVEF). Specimens were cooled at a rate of about 0.3—0.7 °C / min in a medium consisting of DMEM, FBS and DMSO in a ratio of 6:3:1. In the case where an electric field strength of 15 kV/m was used, cell viability was the highest (about 88 %) compared to the HVEF-untreated control and the groups with electric field strengths of 10 or 20 kV/m. The untreated control exhibited altered cell morphology and poor adhesion to the substrate after tha wing.

DENTAL PULP CELLS (MIGRATION FROM THE CRANIAL NEURAL TUBE)

Stem cells derived from the pulp of third molars were cryopreserved in cryoprotective media based on 0.5, 1.0 and 1.5 M ethylene glycol, propylene glycol or DMSO at a cooling rate of 1 °C / min down to –85 °C, followed by immersion into liquid nitrogen [71]. The viability of cells frozen with 1.0 and 1.5 M DMSO was significantly higher compared to other cryoprotectants used in the corresponding concentrations, and made (90.6 ± 8.9) and (91.0 ± 8.1) %, respectively.

B.C. Perry *et al.* [55] cryopreserved the dental pulp stem cells both as a cell suspension and as a part of a whole tooth. The samples were frozen with 10 % DMSO at a cooling rate of 1 °C / min down to –85 °C and then immersed into liquid nitrogen. The difference in freezing of these specimens was that the cryoprotectant was added as a droplet for cell suspension, but whole tooth was saturated with DMSO solution at 4 °C for one hour. The cell viability rate after thawing was 89.5 %. The authors have found no differences between the specimens in their ability to differentiate into osteoblasts and adipocytes. In contrast, E.J. Woods *et al.* [71] have revealed the reduced cell recovery from third molars, cryopreserved with 10 % DMSO (a 2-hour incubation at 4 °C). Cells were isolated from thawed teeth, but after 28 days of cultivation, only 20 % of cells were attached and actively proliferated in culture.

M. Zhurova *et al.* [81] have suggested the presence of intercellular junctions in cell culture contribute to better preservation of human third molar pulp stem cells. In cryomicroscopy experiments, freezing cells as a suspension results in death of about 86 % of cells, while freezing them as a monolayer caused death of about 25.5 %. Under these conditions, the cells were able to express the gap junction protein Connexin-43 and retained membrane integrity, but lost their ability to proliferate.

S.Y. Lee *et al.* [39] have proposed an approach to reduce DMSO concentration in cryoprotective medium for storing dental pulp stem cells. Cryopreservation using a programmable freezer (0.5 °C / min cooling rate) coupled to a magnetic field (0.01 mT) enabled to improve the cell viability. This index for cells frozen with 3 % DMSO and a magnetic field was 73 %, while without magnetic exposure it made 56 %.

N. Huynh *et al.* [34] have demonstrated that cooling rate has a higher impact on dental pulp stem cell preservation than the cryoprotectant concentration. The viability of cells frozen at a controlled cooling rate of 1 °C / min with 5 and 10 % DMSO was almost the same (79.7 and 79.0 %, respectively). In comparison, under rapid cooling (immersion in liquid nitrogen), cell viability was 10.8 and 32.1 %, respectively.

N. Pilbauerova *et al.* [56] have used uncontrolled freezing for cryopreservation of first passage stem cells derived from human dental pulp. Under these conditions, cryotubes with cell samples were kept at –20 °C for 1—1.5 hrs, then transferred to a –80 °C freezer and stored for 6 or 12 months. The cryoprotective medium consisted of complete culture medium supplemented with 10 % DMSO. After thawing, the cells retained the features inherent in intact stem cells such as fibroblast-like morphology, proliferative activity, and expression of 21 phenotypic markers. The cell survival, assessed by trypan blue, was between 88 and 91 %, depending on the low-temperature storage period of 6 or 12 months, respectively.

ENTERIC NERVE CELLS (MIGRATION FROM THE VAGAL NEURAL TUBE)

S. Heumüller-Klug *et al.* [31] have tested several cryopreservation modes for enteric nervous system cells of neonatal rats. The using of cooling rate of 1 °C / min and serum-free medium with 10 % DMSO showed the preservation of about 60 % of cells. Supplementation of cryoprotective medium with FBS at a concentration of 20 and 90 % caused no increase in cell survival (45 and 48.5 %, respectively). Vitrification in the commercial serum-free medium 'StemCell Keep™' (BioVerde, Japan) resulted in complete cell death.

CHROMAFFIN CELLS (MIGRATION FROM THE TRUNK SECTION OF THE NEURAL TUBE)

Cryopreservation of fetal chromaffin cells as aggregates using 10 % DMSO and 10 % serum (1 °C / min cooling rate) enabled to preserve the main phenotypic markers (*e.g.*, tyrosine hyd-

roxylase expression) [67]. Frozen-thawed cells were also capable of forming neurites in response to introduction of nerve growth factor (NGF) into culture medium.

Low cooling rates (0.3—1 °C / min) were found to promote the preservation of cells derived from newborn piglet adrenal glands [9, 53, 65]. To procure a primary culture of adrenal cells, two methods were tested. The first method involved obtaining a cell suspension from the adrenal glands and its cryopreserveation using a cryoprotective medium based on 10 % DMSO supplemented with FBS (1 °C / min cooling rate). This enabled to obtain about 80 % of viable cells, which were further placed under culture conditions [65]. According to the second method, adrenal fragments were cryopreserved in a medium with 10 % DMSO at different cooling rates (0.3, 1, 5, 40 and >100°C/min), and then thawed to obtain cells for further cultivation [9]. The highest cell viability (about 90 %) and cell yield were obtained after using cooling rates of 0.3—1 °C / min. Under these conditions, there were no significant differences between the morphology of post-thaw cells, their ability to adhere, and expression of phenotypic markers chromogranin A and βIII-tubulin from the intact cells [9, 53].

CRYOPRESERVATION OF 3D CULTURES OF NEURAL CREST-DERIVED CELLS

It is known that 3D cultures (spheroids, neurospheres) have several advantages over monolayer cultures, since due to their three-dimensional organisation, cells retain the properties of the organ they originate from. Under these conditions, cells express genes and have a morphology structure close to the natural one, exhibit the features of earlier progenitors, and have physiological characteristics of basic cellular functions (metabolism, cell cycle, cell adhesion, proliferation, differentiation, migration, apoptosis, *etc.*).

The 3D cultures of NCDCs more naturally reproduce the cytological microenvironment of nervous system cells, their mechanical and biochemical features, cell-to-cell neuro-glial connections and signal transmission, *i. e.* they are more realistic models compared to a monolayer [20, 54]. Neurospheres are composed of cells at various stages of differentiation (stem cells, proliferating neural precursors, postmitotic neurons, and glia), as identified by the presence of markers for stem and progenitor cells (nestin, vimentin), neurons (beta-III-tubulin), glial cells (acidic glial fibrillary protein, GFAR), and oligodendrocytes (olig2) [7, 26, 29, 58].

The standard protocol for neurosphere cryopreservation involves the cooling at 1 °C / min rate [18, 43, 79] in a DMSO-based cryoprotective medium [27, 32, 43, 79]. S. Heumüller-Klug *et al.* [31] have compared the results of freezing of the enteric nervous system-derived neurospheres depending on cryoprotective medium composition. It was found that cryopreservation using a cooling rate of 1 °C / min and a cryoprotective medium containing 10 % DMSO with either 20 or 90 % FBS enabled preserving 39.7 and 40.3 % of neurospheres, respectively. Cells were found to retain their properties of excitation in response to specific stimulation. The medium containing 10 % DMSO without serum was less efficient and ensured the preservation of only 27.3 % of neurospheres. Vitrification in serum-free Stem Cell Keep™ medium reduced the number of preserved neurospheres to about 10 %.

Cryopreservation using DMSO at concentrations of 5, 7.5 and 10 % and a cooling mode with 0.5°C/min rate down to –20 °C at the stage I and 1°C/min down to –80 °C at stage II followed by immersion into liquid nitrogen enabled preserving the basic properties of spheroids derived from the primary culture of dorsal root ganglion cells (ability of spheroids to adhere; morphological composition of cells within spheroids and their ability to migrate) [3], although these indices were reduced.

E.M. Plaksina *et al.* [57] have evaluated the cryopreservation modes for spheroids derived from neonatal porcine adrenal cells. These modes used a cooling rate of 1 °C / min and cryoprotective media based on 5, 7, and 10 % DMSO supplemented with 25 % FBS. The characteristics of cryopreserved spheroids, such as the ability to adhere, cell migration and monolayer formation were best preserved when using 10 % DMSO. Under these conditions, the addition of FBS to cryoprotective medium had no significant impact on cryopreservation outcome.

O.Y. Novikova *et al.* [51] cryopreserved spheroids derived from dermal papillae by using

a cooling rate of 1 °C / min, cryoprtective medium containing either 5 or 10 % DMSO and 5 % FBS. After preservation at both cryoprotectant concentrations, the spheroids retained their morphological integrity but had a smaller diameter, which was due to partial cell death after thawing.

Thus, the above studies demonstrate the fact that NCDCs can be successfully cryopreserved within spheroids if low cooling rates (0.5—1 °C / min) and cryoprotectant DMSO at concentrations of 5—10 % are used.

CONCLUSIONS

The multipotency and significant regenerative potential of stem/progenitor NCDCs have led to an increased interest of specialists in their clinical use, especially in personalised regenerative medicine. In view of this fact, it is crucial to develop cryopreservation regimens as an important stage of cell processing along with cell procurement and cultivation. However, at present, the works on NCDCs cryopreservation are scarce.

Here, we attempted to systematize the data obtained during the NCDCs cryopreservation. It is clear that the evolutionarily programmed diversity of these cells and the heterogeneity of their stem/progenitor subpopulations that persist in the adult organism complicate this task. The NC derivatives manifest significant morphological variations depending on final differentiation, therefore, we selected the differences in localization and migration of NCDCs along the rostrocaudal axis as the initial feature for systematization. The analysis of the results allows us to conclude that the NCDCs cryosensitivity depends to a greater extent on the degree of their differentiation than on their origin from a particular part of the NC. In most cases, NCDCs were successfully cryopreserved using low cooling rates (0.5—1 °C / min) and cryoprotective media with DMSO concentrations ranging from 5 to 10 %.

In conclusion, we can note that the *in vitro* and *in vivo* studies demonstrate the success of the developed cryopreservation protocols for various neural crest-derived cells, thus enabling the establishment of cryobanks and expanding their use in clinical practice. Although the NCDCs therapy has great promise, the safety and potential risks associated with this approach should be taken into account. Comprehensive studies of their properties (tumorigenicity, immunogenicity, ability to dedifferentiate, *etc*.) and cryopreservation impact on these and other cellular characteristics are needed before these cells can be used as a primary or adjunctive therapeutic agent.

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КРІОКОНСЕРВУВАННЯ КЛІТИН-ПОХІДНИХ НЕРВОВОГО ГРЕБЕНЯ

Клітини-похідні нервового гребеня утворюються з нейроектодерми на ранніх стадіях ембріогенезу. Внаслідок епітеліо-мезенхімального переходу вони мігрують до периферичних структур та диференціюються у нейрони і глію периферичної нервової системи, меланоцити шкіри, клітини зубної пульпи, нейроендокринні клітини, хрящі та кістки черепа, а також у кілька інших фенотипів. Похідні нервового гребеня мають великий потенціал у регенеративній медицині. Кріоконсервування широко застосовується для довгострокового зберігання біологічного матеріалу, який в подальшому може бути використаний у клінічній практиці. У представленому огляді проаналізовано підходи до кріоконсервування клітин-похідних нервового гребеня, отриманих із різних джерел. Дослідження *in vitro* та *in vivo* демонструють успішність розроблених протоколів кріоконсервування різних клітин-похідних нервового гребеня, що дає можливість створення кріобанків та їх поширеного використання у клінічній практиці.

Ключові слова: кріоконсервування, нервовий гребінь, 2D-культивування, 3D-культивування, клітини спінальних гангліїв, клітини пульпи зубів, меланоцити, шванівські клітини.