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RESEARCH ARTICLE

Translational experience of 28 years of use of the technologies of regenerative medicine to treat complex consequences of the brain and spinal cord trauma: Results, problems and conclusions

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ABSTRACT

The retrospective study summarizes 28 years of cell therapy for neurotrauma of different origin. The four experimental groups were the groups of neurotrama that included traumatic disease of the spinal cord, traumatic disease of the brain and chronic vegetative post-traumatic state. The first group received transplantations of the fetal cells of neural tissue. The second group received the tissue engineering surgery with the transplantation of the fetal cells of neural tissue. The third group were the cases of the bioengineering pasty of the damaged brain tissue; and fourth were the cases of neurotrauma that were treated with the transplantation of the hematopoietic stem cells (HSCs) and hematopoietic precursor cells (HPCs). The long-term follow up proved the cell therapy with HSCs and HPCs to be the safest and most effective.

1 Introduction

Traumatic injuries rate the third cause of death after the heart diseases and cancer [1]. Neurotraumas belong to the most severe injuries and are accompanied by high mortality that ranges from 5.1% to 9.9% [1, 2]. Neurotraumas are not the most frequent and make from 30% to 50% of all cases of traumas [3]. The age of the injured varies from 18 to 45 years, hence, it is the most able-bodied contingent [2, 4]. There is a general tendency for the neural trauma to increase by 2% per year [1]. The main causes of neurotraumas are road traffic accidents, sport exercises, occupational accidents, criminal attacks, combat activity or domestic accidents [5]. Neurotrauma is the most frequent cause for the development of the long-term work incapacity and severe disability [1, 2, 5].

The term of the neurotrauma mostly includes the traumatic injury of the central nervous system (CNS) manifested in the traumatic disease of the brain (TDB) and spinal cord, but also the term covers the traumatic injury of the peripheral nerves and traumatic injury of the vegetative nervous system [1, 2]. Unique achievements of the contemporary neurosurgery and advances of neural intensive care along with successful prosthesing of the vital functions in the cases of severe neurotrauma became the reason for the survival of the patients in the cases that were previously considered fatal [6]. This led to the significant increase of the number of the severely disabled people in the

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population, who have no promise for the full-bodied restoration of the lost functions of the CNS and ability to work, as well as for good life quality. Low effectiveness of the therapy of neurotrauma and impossibility to completely restore after the damage of the brain and spinal functions are conditioned by the absence of medical technologies to restore the damaged neural tissue of the brain and spinal cord [5, 7].

The clinical picture of the disease in the case of neurotrauma involves disorders or loss of the physiological functions of the brain and/or spinal cord which are conditioned by the damage of the specific site of the neural tissue that provides for these functions. Most frequently, the TDB damages the brain functions that provide for the higher nervous activity, and, namely, cognitive functions, intellectual-mnestic and emotional-volitional sphere [1, 2, 8]. The consequences of the neurotrauma accompanied by the brain injury are the main reason for the development of the Walter-Buell syndrome with the dementia outcome. In the case of the spinal cord injury (SCI) mostly the motor and sensitive functions of the body and extremities below the site of injury are damaged, as well as the functions of the pelvic organs (urination, defecation, sexual functions, etc.) [9, 10].

Despite the advances of neurosurgery and pharmacology, no substantial progress in the therapy of the neurotrauma has been achieved in the past two decades. It should be acknowledged that neurologists and neurosurgeons of the world are rather poor therapists of this disease. According to the Human Brain Project (HBP) the annual expenses of the European Union on the CNS injuries treatment totals 80 billion euro [11]. This is closely associated with the medical inability to fully restore anatomy and morphology of the damaged brain and spinal cord to the state that preceded the neurotrauma, and to fully restore the damaged function of the CNS [5]. The efforts of the neuroscientists have not yet led to the success and the search for alternative methods to treat severe neurotrauma accompanied remains topical [4, 12].

In the past years we laid our hopes to find effective therapy for neurotrauma on the technologies of the regenerative medicine that are based on the use of the biomedical preparations of the stem cells (SC), preparations based on the genomic and post-genomic modification of the live SCs and the use of the technologies of tissue engineering and neuroengineering [9, 10, 13]. However, the real role of the SCs in the neurotrauma therapy is not yet finally determined [13]. It can be explained by the absence of the sufficient mass of the clinical data from the cases of such therapy and requisite temporal distance to evaluate the effectiveness and possible long-term adverse effects of using the preparations of SCs. It is considered that so far the obtained statistical material is insufficient for the systemic analysis that could provide estimate of the effectiveness, safety and relevancy of the use of the methods of regenerative medicine for the restoration of the morphology and function of the injured brain and spinal tissue [1, 13].

The goal of the article is to summarize, systemize and analyze our translational experience of clinical use of the different technologies of regenerative medicine in the complex therapy of neurotrauma involving cell therapy, tissue engineering, bioengineering, radio neuroengineering and biomedical cell products of various origin as well as evaluation of their effectiveness and safety in the acute and chronic periods of neurotrauma.

2 Materials and methods

The article is based on our own long-term (28 years) dynamic clinical study of 565 cases of neurotrauma. The first group included 220 cases of neurotrauma that had been treated by the cell preparations obtained from fetal neural tissue of the cephalic vesicles of human embryos at the 12-24 weeks of gestation. The patients were treated in the neurological and neurosurgical department of the 32nd Central Navy Clinical Hospital of the Ministry of Defense of the Russian Federation from August 1989 to July 2002. All biomedical preparations from fetal tissue were prepared for clinical use in the cultural boxes of the Laboratory of Immunohistochemistry of the Department of Fundamental and Applied Neurobiology of the Serbski State Research Center of the Social and Forensic Psychiatry according to our patent for invention RF #2146932 dated November 12, 1998 [14]. All research of the biomaterial and limited clinical trials were performed under the framework of the State Interdisciplinary Program "Neurotransplantation for the Injuries of the Nervous System and Locomotor System"

dated 1989. The program was realized under the supervision of the Scientific Board and Ethics Committee of the Central Navy Clinical Hospital of the Ministry of Defense of Russia, proceedings protocol dated June 15, 1989. The Scientific Board of the Research Institute of Transplantology and Artificial Organs of the Ministry of Healthcare of Russia approved the research in proceedings protocol#18 dated May 16, 1989 and the Ethics Committee of the Research Institute of Transplantology and Artificial Organs of the Ministry of Healthcare of Russia approved the research in proceedings protocol#15 dated May 12, 1989. From 2003 till 2016 the work continued within the framework of the Branch Research Program of the Russian Academy of Medical Science "New Cell Technologies for Medicine". The work was approved by the Scientific Board of Pirogov Russian State Medical University in proceedings protocol#8 dated February 27, 2004, and by the Ethics Committee of Pirogov Russian State Medical University in proceedings protocol#6 dated January 26, 2004. In 2005 the clinical use of the HSCs was approved by the Federal service in Healthcare and Social Development Surveillance of Russia and Registration Certificate FS-2005/026 was issued on June 01, 2005. The Registration Certificate was extended on July 01, 2006, FS-2006/151.

The trial was not registered in any public registries since it was initiated in 1989, when the international trial registration was not required, while in 2005 the basic method was approved for the clinical use.

The distribution of the patients of group#1 is shown in Table 1.

The distribution of the neurotrauma cases of the 1st group by age and gender is shown in Table 2.

The second clinical group involved 48 cases of the SCI (2 females and 46 males) including 2 cases of complete anatomical dissection of the spinal cord. Forty eight surgical interventions for tissue engineering of the spinal cord have been given. The surgeries in tissue engineering of the spinal cord implied laminectomy, meningoradiculomyelolysis, cyst drainage, removal of the cicatrices and commissures, and implantation of composition of the gel and fetal cell preparations. The composition consists of 1-3 mL of the biodegradable heterogeneous biopolymer matrix *SpheroGel*© [15] and the preparation of the fetal nerve cells obtained from the brain of the human embryo (8-12 weeks) or human (12-22 weeks) in the amount of 5×10^6 cells per 1 mL of 0.9% NaCl solution.

The third clinical group included 55 cases (112 records) of neurotrauma who had been received the low-invasive bioengineering plasty of the damaged brain [4]. The group included the cases in which the size of the post-traumatic ischemic injury of the brain

Table 1 The distribution of the neurotrauma cases of the 1st clinical group (allogeneic cells of fetal neural tissue).

No.	Type of neurotrauma	Clinical records	Case numbers	Control group
1	Traumatic disease of the spinal cord	134	102	27
2	Traumatic disease of the brain	136	106	24
3	Chronic vegetative post- traumatic states	19	12	7
4	**Total**	**289**	**220**	**58**

Table 2 Distribution of the 1 group (allogeneic cells of fetal neural tissue) cases by age and gender.

No.	Type of neurotrauma	Males			Females			Control group No. 1 (males only)		
		Number	%	Average age	Number	%	Average age	Number	%	Average age
1	Traumatic disease of the spinal cord	130	44.9	19. 2	4	1.4	26.2	27	46.5	18.8
2	Traumatic disease of the brain	132	45.6	18.6	4	1.4	19.2	24	41.4	19.5
3	Chronic vegetative post-traumatic states	16	5.5	20.4	2	0.7	19.8	7	12.1	26.6
4	**Total**	**278**	**96.1**	**19.4**	**10**	**3.4**	**21.7**	**58**	**100**	**21.63**

did not exceed 5% of the general volume of the brain, and no effect from the preceding cell therapy had been observed for one or two years. The size of the damage was calculated with the densitometry control software in computer tomography (CT). The damage of the neural tissue was considered ischemic if the measurement varied from 15 to 20 the Hounsfield units (HU). The injured brain was operated on according to the technology of the bioengineering plasty that we patented in 2000 in Russia. The technology of the step-by-step restoration of the neural tissue of brain or spinal cord involved the following low-invasive methods of treatment (Fig. 1): programmed regional perfusion of the pharmaceuticals, X-ray methods of angioplasty, stereotactic transplantation of fetal cells of neural tissue, implantation of neurostimulators and technologies of transcranial magnetostimulation (The Russian Federation Patent for Invention of No. 2152038 dated 27.07. 2000) [1, 4].

The clinical translational research also included 345 cases (1973 records) with various organic damages of the CNS that constituted clinical Group 4. Group 4 received long-term in-hospital experimental treatment in the NeuroVita Clinic of Restorative Interventional Neurology and Therapy from September 2002 to March 2017 under the branch program of the Russian Academy of Medical Sciences *New Cell Technologies to Medicine* supervised by Prof. Yaryghin). In 2005 and repeatedly in 2006 the Roszdravnadzor (healthcare surveillance) of the Ministry of Healthcare of Russia issued the first official approval of the clinical use of the preparation of hematopoietic stem cells (HSCs) for neurotrauma. The method of production and use of the cell preparation was patented in Russia (The Russian Federation Patent No. 2283119 dated March 29, 2005) and abroad (WO.2006.102933 International Application No. PCT/EP 2005/008527 05/08/2005) [16]. The characteristic feature of the therapy is that the biomedical cell preparations are prepared from the autologous HSCs and hematopoietic progenitor cells (HPCs) from the leukoconcentrate of the mobilized peripheral blood of the patient. The method to obtain the cell preparation has been described previously [17]. The preparation is administered intrathecally into the subarachnoid space of the spinal cord, or intra-arterially during X-ray program perfusions of the site of the brain plasty, or into the parenchyma of the brain and/or spinal cord during the reconstructive neurosurgical interventions [18, 19]. According to the protocol, the patients received from three to ten

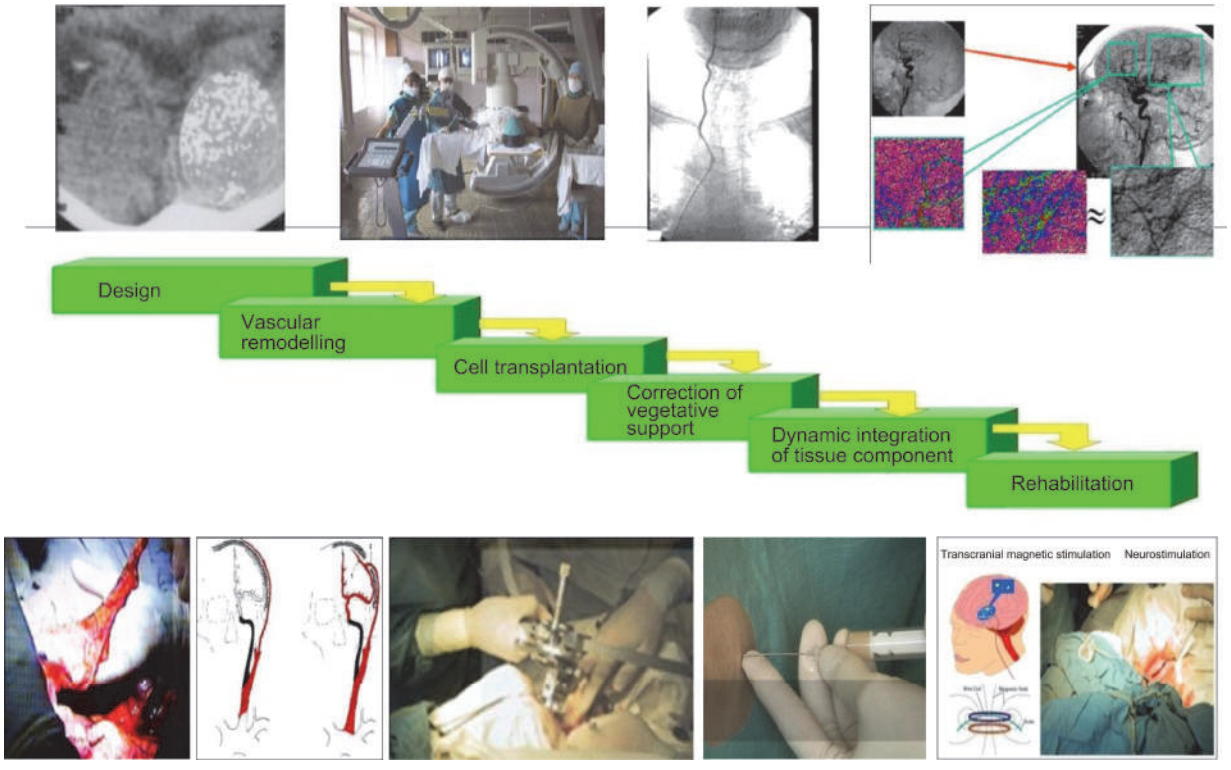


Fig. 1 The stages of the interventional low invasive bioengineering plasty of the neural tissue of the human brain and spinal cord.

transfusions of the cell preparations. Some of the patients with neurotrauma and, namely, 20 cases, received from 32 to 40 transfusions in the course of 8 to 10 years [20]. All cell preparations were prepared, standardized and certified by the laboratory of the Bone Marrow Bank of Blokhin Russian Cancer Research Center.

The distribution of the patients of the Group 4 according to the type of the trauma is shown in Table 3.

Distribution of Group 4 patients by age and gender is shown in Table 4.

As seen from the presented material, the patients of various age groups participated in the study. The age of the control group patients was matched with the research group. However, only men were enrolled into the control No. 1 and No. 2, but we presume that gender did not influence the results of this research.

Group 5 included 52 cases of SCI (5 females and 47 males) including three cases of complete anatomical dissection of the spinal cord. Group 5 received surgical tissue engineering intervention. The intervention implies large neurosurgical reconstructive operation including laminectomy, meningoradiculolysis, cyst draining, removal of cicatricial and commissural tissue, and implantation of 1-3ml of the biodegradable heterogeneous biopolymer matrix *SpheroGel*© and 5×10^6 autologous mobilized mononuclear cells of peripheral blood that contained 102 million HSCs and HPCs (CD34+CD45- HLA DR+).

In ten cases of severe neurotrauma the patented neuroendorposthetic system (the Russian Federation Patent for Invention No. 2394593 dated September 25, 2008) was used [15]. The implantable neuroendoprosthetic system consists of the preparation of the bone marrow mobilized HSCs (CD34+,CD45-) and HPCs (produced by Blokhin Cancer Research Center), neural stem cells (CD 133+) isolated from the olfactory sheath of a nose (produced by Serbski State Research Center of the Social and Forensic Psychiatry) and mesenchymal stromal stem cells isolated from bone marrow of the patient (produced by the Federal Research and Clinical center of the Federal Medical Biological Agency of Russia). All cell material was standardized and certified by the public research institutions. The quality certificates are attached to the case histories of all patients.

The control Group 3 to be compared with the tissue-engineering group included 30 male SCI cases who received the standard neurosurgery for the diagnosed post-traumatic block of the cerebrospinal fluid supply. The surgery included laminectomy,

Table 3 Distribution of the Group #4 (autologous HSCs and HPCs) cases.

No.	Type of neurotrauma	Number of records	Number of cases	Control group No. 2
1	Traumatic disease of the spinal cord	1714	309	88
2	Traumatic disease of the brain	216	31	34
3	Chronic vegetative post-traumatic states	43		5
4	**Total**	**1973**	**345**	**127**

Table 4 Distribution of Group 4 (HSCs and HPCs) cases by age and gender.

No.	Type of neurotrauma	Males			Females			Control group No. 1 (males only)		
		Number	%	Average age	Number	%	Average age	Number	%	Average age
1	Traumatic disease of the spinal cord	253	73.3	34.5	56	16.2	42.4	88	69.2	33.8
2	Traumatic disease of the brain	23	6.6	28.6	8	2.3	31.2	34	26.7	39.5
3	Chronic vegetative post-traumatic states	4	1.16	27.4	1	0.3	19	5	3.93	34.6
4	**Total**	**280**	**81.2**	**30.17**	**65**	**18.8**	**30.87**	**127**	**100**	**36.97**

meningoradiculomyelolysis, cyst draining and dura mater plasty. Average age of the group was 24.3 years. All patients signed the informed consent.

Neurotrauma group that received HSCs and HPCs included 16 male patients (average age 48.2) who received distant multiwave radiobioengineering (DMW RBE). The method has been described in details in the *Journal of Neurorestoratology* [21] and patented in the Russian Federation. Three patients of this cohort were in post-traumatic chronic vegetative state.

All patients with different clinical manifestations of the neurotrauma were followed up for 3 to 18 years and passed in-hospital check-ups every three months. The check-up included neurological examination and contrast-enhanced magnetic resonance imaging (MRI) of the brain and/or spinal cord. The tractography of conducting pathways of the brain and spinal cord on the basis of diffuse tensor MRI was done in 20 cases. All SCI cases had electroneuromyography (ENMG), ENMG with somatosensory evoked potentials, passed tests according to different measurement scales (ASIA, FIM), X-ray control of knee and hip joints meeting the demands of the research protocol approved the Scientific Board and ethics Committee of the Pirogov Russian State Medical University (RSMU) and international protocol of the cell therapy and tissue engineering IMITE (Switzerland). All intermediate and final reports have been timely presented to the RSMU head office, Roszdravnadzor and Ministry of Health Care of the Russian Federation.

3 Results

By now our team has gathered large (16 000 transplantations of different cell preparations to 5506 cases of various CNS diseases) and long-term (28 years) experience of using biomedical cell preparations in the cases of neurotrauma, and it seems time to present it to the scientific community, to summarize and to give primary analysis of the effectiveness and safety of their administration. First, we analyzed the appropriateness and feasibility of using different strategies of administration of the stem cell preparations and technologies of regenerative medicine in the various neurotrauma cases. Our studies showed that in 94.2% the administration of the cell preparations for severe neurotrauma was not conditioned by the ineffectiveness or low effectiveness of the conventional methods of severe neurotrauma treatment. Depending on the size of the organic damage of the brain or spinal neural tissue and severity of the clinical condition, various strategies involving biomedical cell preparations have been applied in the treatment.

Distribution of the patients depending on the strategy of using the regenerative medicine technologies (conservative, surgical or combined) is shown in Table 5. As seen from Table 5 the main strategy of clinical application of the preparation of SCs was the cell therapy, that constituted 72.4%; tissue engineering was used in 13.8%. And in 13.7 cases we combined tissue engineering of the spinal cord with the cell therapy.

The evaluation of the effectiveness of the cell therapy depends on the localization of the site of injury in the brain and spinal cord and severity of the clinical manifestations, as well as on the goals of SCs administration. Analysis of the effectiveness in various types of neurotrauma is shown in Table 6. As seen from the data of the table the effectiveness of the SCI made 47.6%, while in 15.3% the therapy was

Table 5 Distribution of the neurotrauma cases depending of the strategies of regenerative medicine.

No.	Type of neurotrauma	Conservative strategy (cell therapy)		Surgical strategy (tissue engineering)		Combined strategy (tissue engineering + cell therapy)	
		Number	%	Number	%	Number	%
1	Traumatic disease of the spinal cord	220	91.6	47	95.9	12	44. 4
2	Traumatic disease of the brain	14	5.8	2	4.1	15	55.6
3	Chronic vegetative post-traumatic states	6	2.5	—	—	—	—
4	**Total 316 cases**	**240**	**100**	**49**	**100**	**27**	**100**

Table 6 Effectiveness of the long-term therapy with HSCs and HPCs for different types of neurotrauma.

No.	Type of neurotrauma	Deterioration		No effect		Effective		Highly effective	
		Number	%	Number	%	Number	%	Number	%
1	Traumatic disease of the spinal cord	220	91.6	47	95.9	12	44.4	53	15.3
2	Traumatic disease of the brain	14	5.8	2	4.1	15	55.6	3	0.86
3	Chronic vegetative post-traumatic states	6	2.5	—	—	—	—	1	0.3
4	**Total 346 cases**	**6**	**1.7**	**94**	**27.1**	**189**	**54.6**	**57**	**16,47**

highly effective. Effectiveness of the therapy was evaluated ASIA (American Spinal Injury Association) and FIM (Functional Independence) scales, as well as by EEG and ENMG with somatosensory evoked potentials. The results were judged highly effective if one of the damaged functions, such as walking, bowel and bladder function, sensation, was restored completely or close to it.

The diagram of the effectiveness of restoration of the injured spinal cord is represented in Fig. 2 and makes 57.4% in SCI. Meanwhile general effectiveness of the therapy that involved intrathecal transfusions of the cell preparations for the neurotrauma of the brain and spinal cord is 54.6%. Comparison with the effectiveness of the therapy in control Groups 1 and 2 showed that the treatment involving cell therapy is more effective (see Tables 7 and 8). Comparative effectiveness of the complex therapy of neurotrauma with cell preparations is higher than the therapy that uses allogeneic (fetal) cell systems.

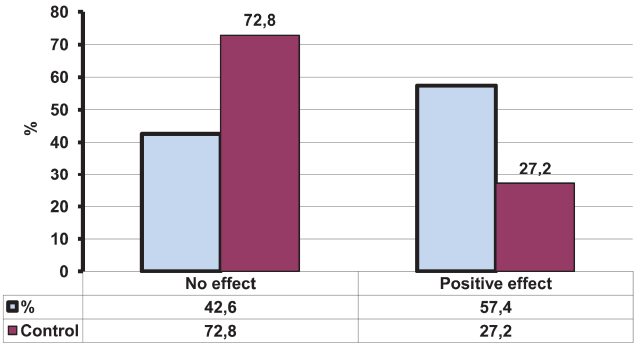


Fig. 2 Four years follow-up of the SCI patients after administration of the autologous HSCs and HPCs (after 4 years).

The results of our early studies of the application of allogeneic (fetal) cell preparations varied from 35% to 46% [2]. In this research we showed that the therapy that involves the technologies of the regenerative medicine is effective in 55.4% and highly effective in 15.3%. The details are presented in Table 9. The effectiveness of the restoration of sensation in the patients with SCI is shown in Fig. 3. The effectiveness of the restoration of the functions of spinal cord was confirmed by the objective data (Figs. 4 and 5).

Altogether, a hundred patients have undergone the tissue engineering of the spinal cord, the restoration of the spinal cord with heterogeneous biodegradable *SpheroGel*© matrix and allogeneic (fetal) cell preparations was done in 48 cases, and the tissue engineering with the *SpheroGel*© matrix and autologous stem cells (HSCs and HPCs) was done in 52 cases. Using the

Table 7 Effectiveness of the conventional therapy of the control group 1 in different types of neurotrauma.

No.	Type of neurotrauma	Deterioration		No effect		Effective		Highly effective	
		Number	%	Number	%	Number	%	Number	%
1	Traumatic disease of the spinal cord	—	—	27	46.6	—	—	—	—
2	Traumatic disease of the brain	—	—	22	37.9	1	—	—	—
3	Chronic vegetative post-traumatic states	1	1.7	7	12.1	—	—	—	—
4	**Total 58 cases**	**1**	**1.7**	**56**	**96.6**	**1**	**1.7**	-	-

Table 8 Effectiveness of the conventional therapy of the control Group 2 in different types of neurotrauma.

No.	Type of neurotrauma	Deterioration		No effect		Effective		Highly effective	
		Number	%	Number	%	Number	%	Number	%
1	Traumatic disease of the spinal cord	2	1.5	58	45.6	28	22.0	—	—
2	Traumatic disease of the brain	—	—	22	17.3	12	10.4	—	—
3	Chronic vegetative post-traumatic states	2	1.5	3	2.3	—	—	—	—
4	**Total 127 cases**	**4**	**3.4**	**83**	**65.4**	**40**	**31.5**	—	—

Table 9 Effectiveness of the long-term therapy with allogeneic (fetal) preparations of neural tissue for different types of neurotrauma.

No.	Type of neurotrauma	Deterioration		No effect		Effective		Highly effective	
		Number	%	Number	%	Number	%	Number	%
1	Traumatic disease of the spinal cord	6	2.01	62	21.5	36	44.4	30	21,18
2	Traumatic disease of the brain	3	1.04	51	17,7	61	55.6	21	7,29
3	Chronic vegetative post-traumatic states	1	0.34	10	3,47	3	1.15	4	1,38
4	**Total 288 cases**	**10**	**3.47**	**123**	**42,7**	**100**	**34.7**	**55**	**19.01**

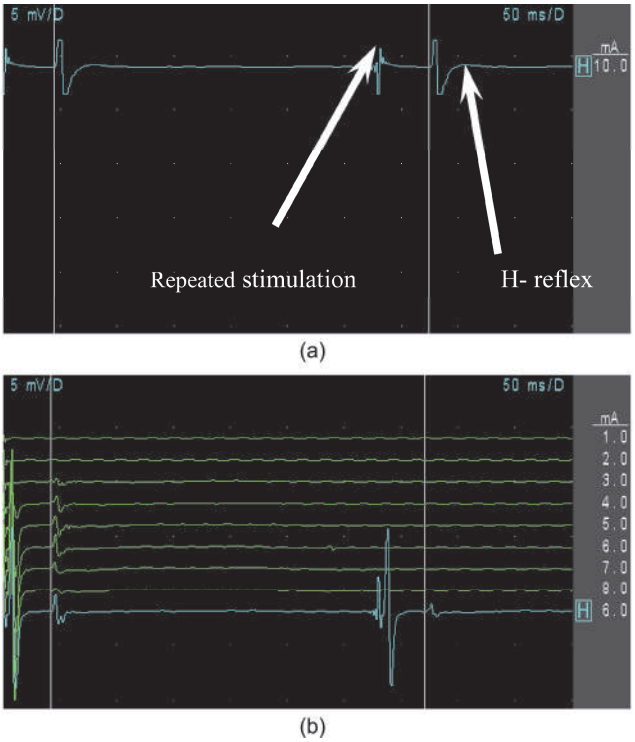


Fig. 3 Restoration of suprasegmentary brain control over spinal reflexes after administration of the autologous HSCs and HPCs. (a) Absence of H-reflex habituation in patient R. with absent motor functions before treatment. (b) Increase of H-reflex habituation in patient R. with improved motor functions after autologous HSCs and HPCs therapy.

technologies of tissue engineering in the SCI our team restored the damaged spinal cord almost in all patients to a certain extent, which is confirmed by the results of the follow up, including CT-myelography, MRI, ENMG. However, restoration of the disordered functions was not regularly observed. Usually, the first results after the surgery have been noted 18-24 months later, and sometimes even 3 to 4 years later. Most frequently, the motor functions of the extremities were restored, and, namely in 42.6%, while the sensation was restored in 19.5%. However, the functions of the pelvic organs were restored in 56%

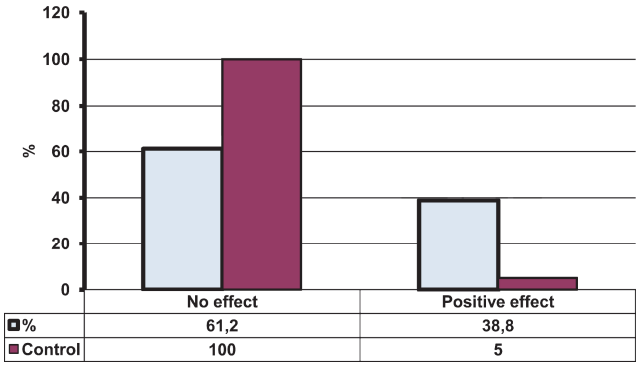
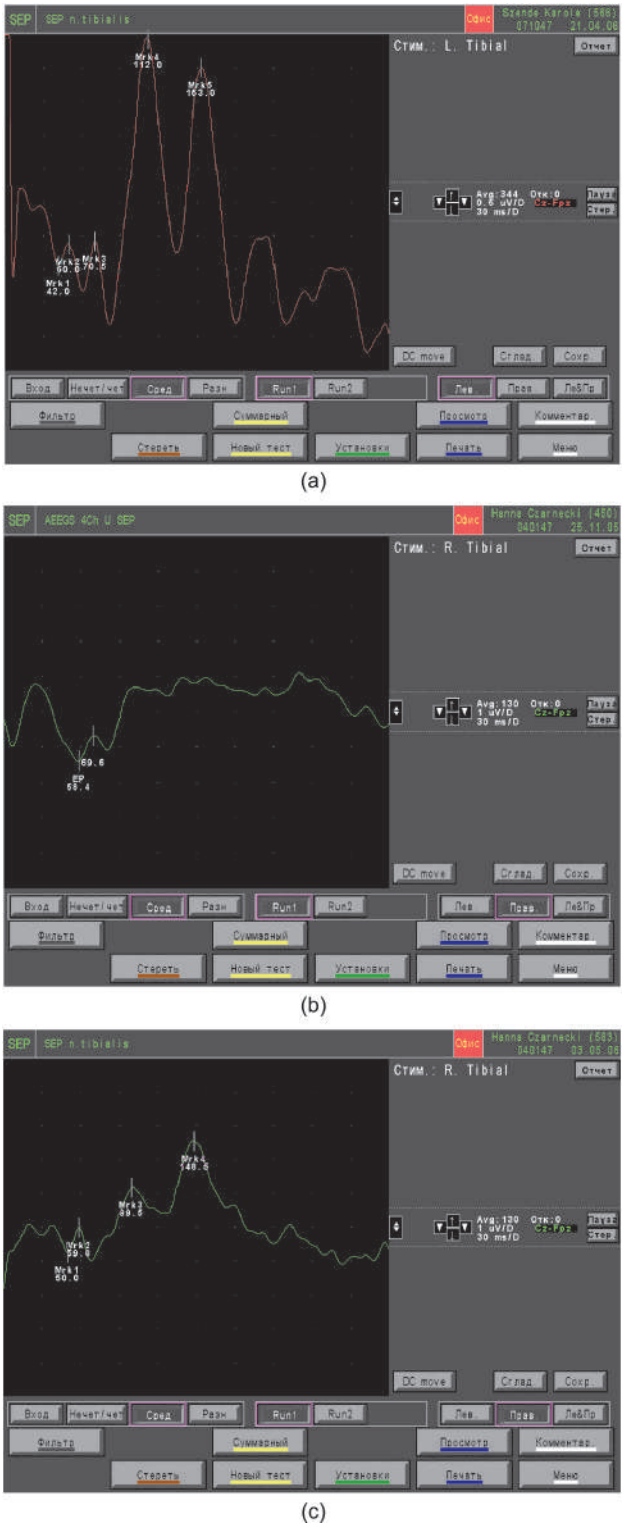


Fig. 4 General efficiency of sensation restoration after administration of the autologous HSCs and HPCs (after 4 years).



(a)

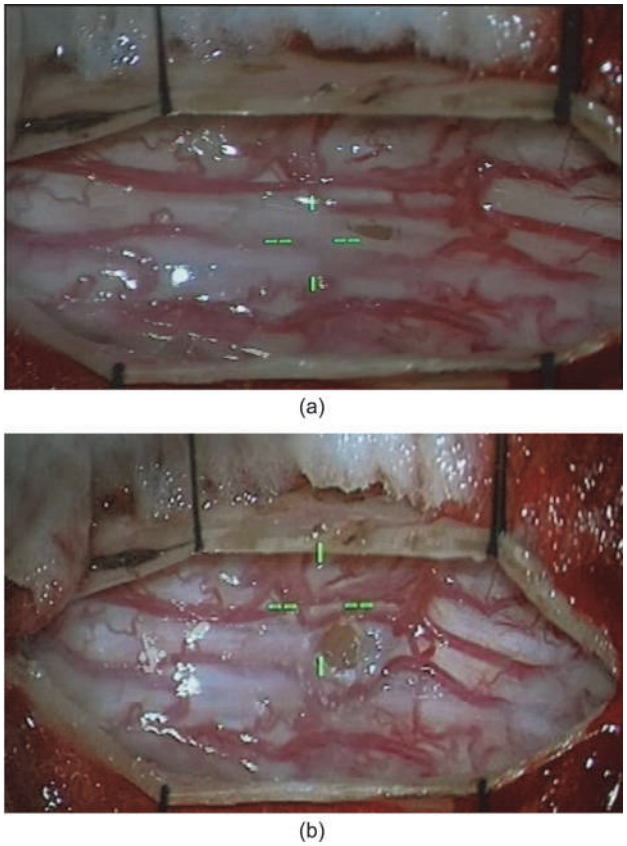
(b)

(c)

Fig. 5 Changes of ENMG somatosensory evoked potentials (SSEP) in patient Ch. with C5-C6 SCI during MAHSC therapy A- SSEP – normal variant B - SSEP patient Ch. before therapy C- SSEP patient Ch. after MAHSC administration in 6 months.

of the cases. First, these patients started to partially control the urge to urinate and defecate, which was confirmed by the urodynamic tests, and 31% of the cases observed controlled urination by year 5 or 6 post surgery.

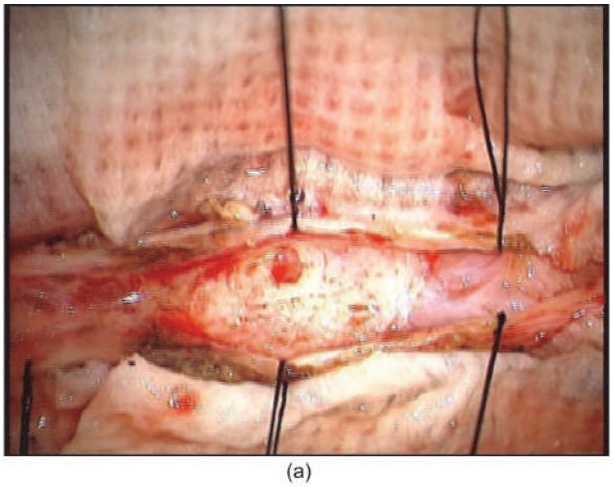
The example of the tissue engineering surgery of the spinal cord is shown in Fig. 6, where the matrix and cells were placed into the drained cyst of the spinal cord and closed with the biopolymer cover *ElastoPOB* to form endoprosthesis and to protect it from the aggressive environment (CSF, blood, etc.). The anatomical-morphological structure of the damaged spinal cord was restored in 10 cases, although in some cases the injury was 10 to 15 years old (Fig. 7), so that the conductance of the electrical impulse through the damaged area was restored at least partially (Fig. 8). The effectiveness of the tissue engineering and bioengineering varied from 41% to 49.6%. However, our team appeared unable to enhance the general effectiveness of the technologies of regenerative medicine over 56% despite the variety of the applied cell preparations. Still, the administration of the cell preparations appeared safe. We did not observe



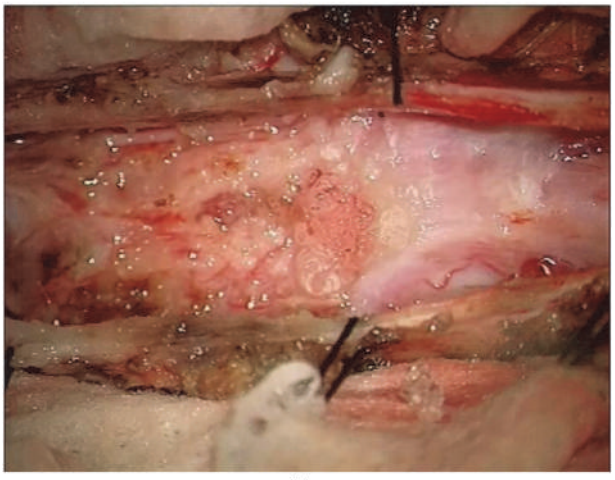
(a)

(b)

Fig. 6 Implantation of the matrix and stem cells into the cyst: (a) the cyst of the spinal cord is opened and drained, and (b) external appearance of the spinal cord and implant.



(a)



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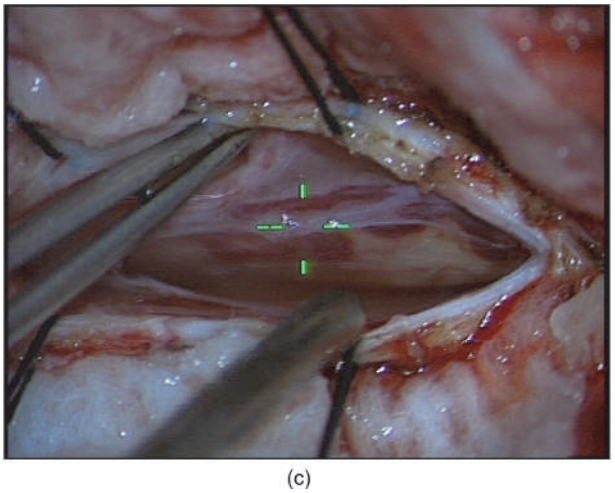


(c)

Fig. 7 The external appearance of the spinal cord of the patient S. During the tissue engineering surgery in 2007 at C5-C6 level. (a) Cicatricial and commissural degeneration of the spinal cord, calcification in the site of injury. (b) The state of the spinal cord after removal of the calcification, menigoradiculomyelolysis and implantation of the matrix and stem cells into the cyst. (c) State of the spinal cord during repeated surgery in 2009: completely restored blood supply, anatomical structure and electrical conductance in the site of injury.

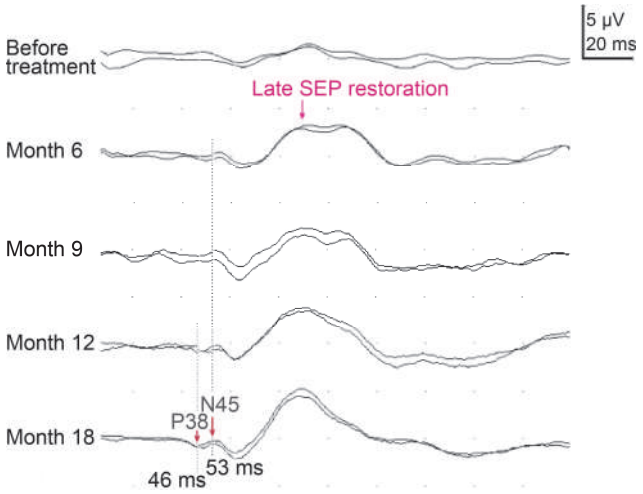


Fig. 8 The dynamics of SEP elicited by stimulation of left tibial nerve of C5 level SCI patient. The stem cell treatment was started 4 years after injury. Note the restoration of short-latency components – firstly N45 and then P38. The simultaneous latency reduction of the appeared components is seen. Note also the increase of late components amplitude.

significant difference in the types of cells (HSCs, NSCs, MSCs) combined with tissue engineering. In all cases the long and technical sophisticated microsurgery was tolerated well, no lethal outcomes, all patients feel good until now. The change in the state of the SCI patients was measured by the ASIA and FIM scales. The ASIA impairment scale characterizes the degree of the disability very well, but is low effective in the analysis of the dynamics of restoration of the disordered functions of the spinal cord, which had been previously noted [1, 2]. We could not confirm the clinical effect of the tissue engineering in 16 patients who received fetal biomaterial and in 5 patients with SCI who received the therapy with autologous cell systems.

The results of the use of the methods of radioneuro-engineering for neurotrauma appear biased, because the neuroengineering was administered to the patients with extremely severe neurotrauma, when the tissue engineering and cell therapy appeared ineffective. The detailed characteristics of the method and clinical examples of its realization have already been published [22]. It should be noted that in each of four cases of radioneuroengineering administration, we observed the positive result of the clinical improvement manifested in restored consciousness, improved cognition and restored intellectual-mnestic activity of

the damaged brain. Mostly, radio neuroengineering was given in the cases of post-traumatic chronic vegetative states or obvious residual organic damage of the brain. The technology requires separate discussion as related to the indication s and methods to evaluate effectiveness.

Thanks to this long-term experience we can claim that the complications after administration of the preparations of autologous stem cells are almost absent, especially if compared to the pharmaceuticals. There were only two cases when administration of the fetal cell biomaterials led to the vital complications in the patients with acute SCI. The complications manifested in the development of acute autoimmune encephalomyelitis. In one case our team successfully managed the situation with adequate pharmacotherapy, while in the other case we had to face the lethal outcome conditioned by the severity of the trauma (incompatible with life) and the diagnosis was verified in the autopsy as incidental finding. Intrathecal administration of the fetal biomaterial in the cases of neurotrauma demonstrated possibility of the development of distant immune reaction to the introduction of the foreign cells [2]. Despite a well known and much discussed immune privilege of the fetal materials, 26 patients demonstrated various vague host versus graft reactions that we successfully reduced by the corticosteroids and desensibilization drugs. In five cases we have observed significant toxic complications associated with the irritation of the brain membranes and accompanied by the episodes of clouded consciousness and stupor. Adequate intensive care coped with the symptoms quickly. Our examination of these facts showed that the toxic complications have been caused by the cell preparations that were insufficiently washed from the biologically active media in which the cells were cultured or cryopreserved. Administration of the autologous cell biomaterial did not lead to such adverse events. All complications of the tissue engineering and low invasive bioengineering interventions were conditioned by the flaws of the surgical technique and not the effect of the cell suspensions. As far as there is no surgery without complications, the development of these adverse events was predictable and it was found in 5.6% cases. None of the cases of adverse events was associated with the administration of the cell preparations.

4 Discussion

Our long experience of using various cell products in the clinical practice permits the statement that the therapy of neurotrauma with the technologies of regenerative medicine is more effective than similar therapy without them. Undoubtedly, cell therapy involving autologous HSCs and HPCs is the most effective treatment of the neurotrauma [16, 23]. We are deeply convinced that administration of the autologous HSCs and HPCs in the leukoconcentrate of the mobilized mononuclears of peripheral blood for traumatic disease of the brain and spinal cord must become the standard therapy both in early restorative period after the trauma and in the late restorative period. Leukaconcentrate of the mobilized mononuclears provides a 3D basis in which the HSCs and HPCs function.

The court makes the king and the effectiveness of the HSCs and HPCs is conditioned by the mononuclears. They form the cell cluster which provides for the regulatory effect of the signals of bone-neural niche in the HSCs, HPCs and MSCs of the mononuclear leukaconcentrate. This fact has been discussed in our previous works [8, 22]. This is why administration of the isolated HSCs after bone marrow transplantation leads to the lethal outcome after the high-dose therapy, while administration of the HSCs together with the leucoconcentrate provides for the restoration of the hematopoiesis.

Administration of the cell preparation in the acute stage of brain and spinal cord injury seems impractical. The criteria to administer cell therapy should include reduction of the acute events of post-traumatic edema and swelling of the brain and spinal cord, resolution of the sites of ischemia of neural tissue (densimetric CT-control of the ischemic sites) and complete restoration of the vital functions of the body. Administration of the cell preparation before four to six weeks pots-injury is impractical and ineffective as almost all stem cells and precursors will be eliminated by the post-traumatic inflammation of the neural tissue.

Our team has consciously refused from the clinical use of the fetal material to treat neurotrauma. It is conditioned not only by the moral, ethical, religious and legal issues, or, even by the possibility of immunological responses and complication, but also by the

pseudoscientific character of administration of this cell material in the clinical practice. Administration of the fetal material to neurotrauma can give an incredibly fantastic result and demonstrate all the miracles of regeneration of the damaged CNS in one of the hundred cases. But you will never be able to repeat this result, because the material to obtain the therapeutic dose of the fetal cell preparation is gathered from three to four donors, making impossible to repeat this combination of tissue components. Preparation of the fetal cell material from the line of the embryonic stem cells (ESCs) requires production and storage of a huge amount of new lines of the ESCs which is expensive and ineffective. This disadvantage of the fetal material surpasses all its advantages.

Another important issue of the cell therapy of neurotrauma is to predict the scope of restoration of the lost function after the restoration of the site of injury in the brain or spinal cord. Morphofunctional integrity of the topical focus of the traumatic injury of neural tissue is the canonized view on the localization of the function on the brain/spinal cord in contemporary sciences and is not entitled to be discussed. It is universally acknowledged that specific local organic morphological defect of the brain and spinal cord is the reason for the disorders or absence of the main functions of the nervous system and irreversible disease of the higher nervous activity. All atlases of the topical diagnostics of neural diseases and CNS injuries rely on this notion. The fundamental basis and morphological mechanism of the clinical development of the syndromes in neurotrauma is organic defect of the neural tissue of brain or spinal cord which is accompanied by the disorder or loss of the function. Hence, the main dogma of contemporary neuroscience assumes necessity of the anatomical-morphological restoration of the damaged neural tissue, and it is supposed that the function will restore on itself, automatically, to the same level as prior to the injury. However, the decades of our research shows that it is possible to restore the neuromorphological organization of the site of injury of the brain/spinal cord with the cell therapy, but it does not equal to the restoration of the same functional of the neural structure that it used to have before the injury in 99%. The process of regeneration and restoration of the anatomical structure in the site of injury in the presence of the stem cells is *chaotic and uncontrolled by the CNS and vegetative nervous system and the outcome cannot be predicted*. The synaptogenesis in the site of injury is also random and unsystematic. Uncontrolled vasogenesis establishes the conditions of misdistribution of capillaries, arterioles and venules, so that they are abundant in one place and lack in the other. Fusion of the injured cells of the neural tissue with the genetic material of the stem cells is also non-systemic and unlimited, thus forming metabolic cell imbalance in the reconstructed tissue.

The observed delay in the first signs of restoration after the surgery seems to be explained by the time required for new axons to grow and to establish the bridge or bypass between the stumps. Even an infant's neural system requires about a year to learn walking, while in the case of injury we have to deal with the chronically disordered patterns and vast damage.

Therefore, the stem cell stimulation of the local neuroregeneration results in the new anatomical-morphological structures that are similar but not identical to the previous tissue. Moreover, these new structures do not have functions; they only have the potential to develop the equivalents of these functions. None of our patients restored the same movements that were typical to them before the injury. No one restored the same gait or full sensation; there were always some areas of anesthesia or hypoesthesia left. The long-term rehabilitation after neurotrauma results lead to the formation of the equivalents of the previous functions, and not the functions. Consequently, it should be taken into account that *neurorestoration cannot restore lost functions, it can only develop the equivalents of the functions*. Hence, the patients and their relatives should be warned that the cells are not able to restore the damaged function in the full scope. The restoration of exactly the same functions of the CNS is not possible even in theory, and even the approaches to it are not clear.

So far, it is not clear how the purposefully assembled tissue of the specific damaged site of neural tissue can be achieved in the way as it happens in every human during embryogenesis and ontogenesis of the tissues and organs. Obviously, the stem cells fulfill the same function of the catalysts and generators of the

regenerative and reparative process in the organ in the ontogenesis and embryogenesis, but in the case of non-systemic reparation and artificial neurorestoration there should exist some "physical" program of the tissue assembling under the effect of the SCs. However, we do not have such a program, and the ways to develop it seem very obscure.

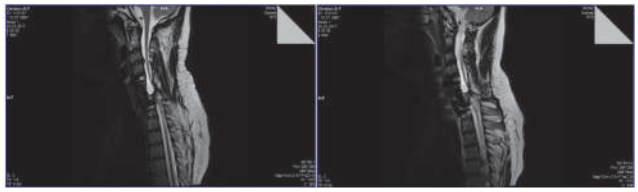
The pathomorphological basis for the complications in the acute neurotrauma is usually provided by the proliferative process in the membranes and matter of the brain and spinal cord, gliosis and atrophy of the brain substance, internal and external hydrocephaly etc. In the long-term period (over 1 year) in the morphological structure of the organic defect of the damaged neural tissue lies development of the cystic, commissural and/or atrophic process [17]. Three years post the neurotrauma onset, the role of etiological factor becomes insignificant and prognostically irrelevant, as we deal with the universal organic process of the reparation of the neural tissue injuries. We came to the conclusion that it would be more correct to name neuortrauma the disease of the injured brain/spinal cord, and artificially activated processes of neuroregeneration are non-systemic and opposing the physiological processes of organogenesis in the embryogenesis and ontogenesis.

The neurotrauma is more fearful with its consequences than with the initial manifestations. However, the best effects of neurorestoration of the consequences of the brain/spinal cord injury have been observed in 76.3% of the cases of neurotrauma of three and more years old. So the best results were received in the therapy of the long-term consequences of the brain/spinal cord injury. Ten or twenty year old injuries must not be contraindications for the cell therapy. Neural tissue of the damaged brain and spinal cord undergoes degeneration that manifests in the cystic and cicatricial metamorphoses. Three years are the period when the damaged neural tissue is able to regenerate using its own sanogenetic resources. This period provides maximally possible restoration of the morphological features of the tissue and/or permits adaptation of the neural tissue to the newly appeared organic defect. All functions of the brain and spinal cord that were disordered due to the formation of morphological defect and failed to restore for the next three years, will hardly restore in the next years independently. However, separate cases of self-restoration of the CNS functions have been described even after 20 years of the chronic vegetative state. Consequently, our experience of using the methods of the regenerative medicine even decades post injury gives hope for positive effect.

One of the most important issues that we noticed during our longitudinal study is the situation of a complete "cognitive dissonance" between the morphology of the damage and the function of the damaged site. We detected two main variations. The first type of "cognitive dissonance": in 15 cases of the 5 year old injury and older the neural tissue was almost absent between the distal and proximal ends (MRI, CT, CT-myelography confirmed) and the gap between the ends of the spinal cord was 2cm large and filled with cicatricial-comissural formation. However, two to five administrations of the cells led to the restoration of the function of walking and almost complete restoration of the pain and temperature sensations which was accompanied by new ENMG activity. Similarly, the MRI after the tissue engineering of the complete anatomical dissection of the spinal cord finds no restored spinal cord, while only tractography shows some single lateral fibers, and these appeared enough to provide almost normal functioning (Fig. 9).

The second variation of the "cognitive dissonance": three patients after tissue engineering of the spinal cord almost completely (confirmed by MRI and visually) restored the damaged anatomic-morphological structure and even the conductance of the nervous impulses through was close to normal according to the ENMG. But the function of the spinal cord that depended on the damaged area was not restored. It can be illustrated by the case with the patient S. when the tissue engineering surgery of the spinal cord gave no effect. The repeated surgery showed almost complete anatomical restoration of the site of injury (Fig. 2(b)) and establishment of the close to normal neurophysiologic bridge in the intra-operative ENMG. However, the patient failed to restore the function even 6 years post surgery.

All this gives us evidence that our understanding of the information traffic in the brain and spinal cord



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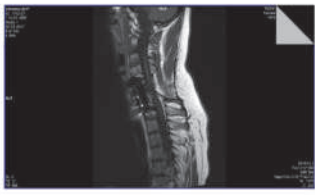

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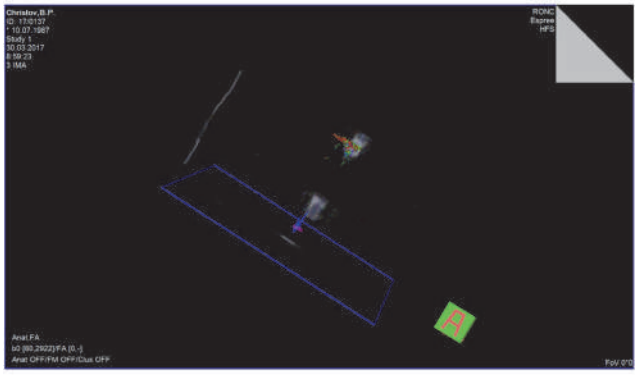

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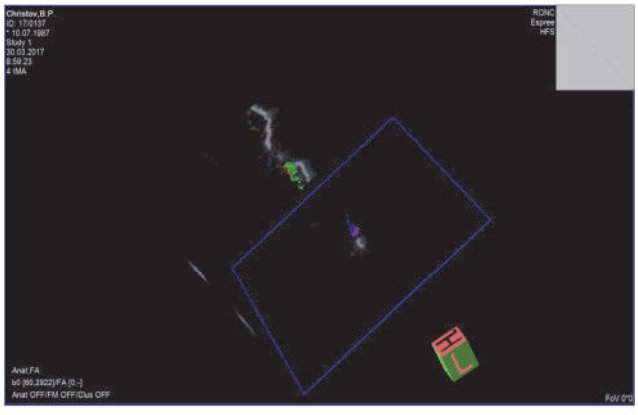

(d)

Fig. 9 Magnetic resonance imaging of the cervical spine three years post tissue engineering in patient C. MRI Conclusion: SCI, late period. Consequences of the compressive-comminuted fracture of C6 and C7 vertebrae, state post tissue engineering surgery. Corporectomy of C6 and C7. Complete dissection of the medullar substance of the spinal cord at the C6-C7 level with dural sac conductance disorder and MR-signs of subatrophic changes of the medullar substance of the intact segments. (a) T2 mode. (b) T1 mode. The tractography showed that the integrity of the medullar substance at C6-C7 level is disordered for 1.98 cm (while in 2013 the size of dissection was 1.14 cm×1.08 cm) but separate neural fiber bands (c, d) along lateral contours are seen. The CSF canal is obstructed.

is extremely limited. The neuroscience community is convinced that if neurosurgeons develop the technology to restore the connections of the neural tissue, they will restore the anatomy of the injured spinal cord, provide for the impulse conductance through the damaged axons in the site of injury and it will automatically lead to the restoration of the function of walking after long neurorehabilitation. Same approach underlies the fantastic project to transplant the head from one human to the other. Our experience says it is a large scientific error. Obviously, to date it is quite possible to fuse the cervical vertebrae with titanic plate or orthopedic construction, to stitch the muscles and vessels between the donor and host tissues. However, the greatest challenge is the restoration of the functions of the spinal cord after technical connection of the damaged ends of the spinal cord to provide motor, sensation functions and the function of bowels and bladder. Our experience permits us saying that the restoration of the function below the site of injury will not occur, as new anatomy does not equal to old functions of the CNS.

Summing up, we presume that the main accent of contemporary research of the stem and precursor cells should be given to the development of new classes of cell preparations with specific properties, which should follow the trends of molecular biology and molecular medicine, rely on genome and whole transcriptome research of gene expression and involve the post-genome research of proteome and secretome of the cell preparations.

Cell targeted therapy opens new horizon for both regulation and maintenance of effector functions of the damaged cells and vessels of the neural tissue.

5 Conclusion

Hence, twenty eight years of the cell therapy of neurotrauma led us to the conclusion that use of the autologous preparations of the HSCs and HPCs in the complex treatment of the neurotrauma is safe and effective. The best effect cell therapy of the brain and spinal cord injury is observed when the size of the defect is insufficient. Use of the fetal cell preparations is not recommended due to the impossibility to repeat and to enhance the achieved clinical effects. Administration of the autologous stem cells to treat

severe neurotrauma is scientifically and clinically grounded and evaluated but he neuroscientists as the key biotechnological engine and catalyst of the process of regeneration in the damaged spinal and brain tissue. The HSCs and HPCs are able to promote regeneration in the brain and spinal cord by paracrine effects, new synapses and by fusion of the stem cells with damages neural cells. Restoration of the morpho-functional defect in the brain and spinal cord injury through low invasive bioengineering of the brain and spinal cord is feasible and possible, but use of such technologies of the regenerative medicine must be well grounded due to high risk of the complications.

Disclosure

The author declares no competing interests.

References

[1] Bryukhovetskiy AS. *Spinal Cord Injury: Cell Technologies in the Treatment and Rehabilitation*. (in Russian). Moscow, Russia: Prakticheskaya Meditsina, 2010.

[2] Bryukhovetskiy AS. Transplantatsiya Transplantation of neural cells and tissue engineering of the brain and spinal cord in neural diseases. (in Russian). Moscow, Russia: ZAO Klinika vosstanovitelnoy interventsionnoy nevrologhii i terapii NeuroVita; 2003.

[3] Bryukhovetskiy AS, Lavrentyev A, Zaisev A. Cell neurotransplantation in the complex treatment of damage spinal cord. Presented at the 4th International Conference High Medical Technologies in 21st Century. Spain, Benidorm, 2005.

[4] Bryukhovetskiy AS, Zubritskiy VF, Chekhonin VP, et al. The method of bioengineering to restore the functions of the brain/spinal cord. The Russian Federation №2152038 RU.

[5] Zhang C, Feng S, Hu N, et al. Osteopontin induces the extension of epidural fibrosis into the spinal canal (Letters to the Editor). *Pain Physician*. 2015, **18**: E9–E99.

[6] Abdelfatteh M, Mohamed B, Moncef BR, et al. Chapter 12. Spinal Cord Injury. In *Neurorestoratology*. Huang H, Raisman G, Sanberg PR, Sharma HS, editors. New York, USA: Nova Science Publishers, Inc, 2015.

[7] Bryukhovetskiy AS. Information communicative organization of brain and its functional principles. Presented at IANR VII&1st SCSI with 11th GCNN & 2nd IFNR Conference, Mumbai, India, 2014 February 27–March 1.

[8] Bryukhovetskiy AS, Avdeykin SN, Evseyev NG. Complications and specific features of the therapy of spinal cord injury with mobilized autologous haematopoietic stem and progenitor cells. Presented at the 21st Meeting of European Neurological Society, Lisbon, Portugal. 2011 May, 28–31. *J. of Neurology*. 2011, **258**: Supplement1.

[9] Bryukhovetskiy AS. Mobilized autologous stem cells in the SCI treatment. *Journal of the Neurological Sciences*. 2005, **238**: S43.

[10] Bryukhovetskiy AS, Dolgopolov R, Pimenov R, et al. PBSC mobilization and collection in patients with traumatic spinal cord. *Bone marrow transpl*.2005, 35, Suppl.2.

[11] Bryukhovetskiy AS. Human Brain Theory. Information-Commutation Device of the Brain and Principles of its Work and Modeling. NewYork: Nova Science Publisher, 2016.

[12] Bryukhovetskiy AS. Epigenetically reprogrammed cell systems of hematopoiesis precursor: From cell therapy to neurorestoration and neuroregulation of brain and spinal cord cells. Presented at *IANRAC III*. Beijing, China, April 23–25, 2010.

[13] Bryukhovetskiy AS. Mobilized autologous stem cells from peripheral blood for the treatment of spinal cord injury (SCI). Efficacy and safety assessment. Presented at the 13th Congress of the European Federation of Neurology Societies. September 11–15, 2009; Florence, Italy. *Eur J Neurol*. 2009, 16(Suppl 3): 302.

[14] Chekhonin VP, Dmitriyeva TB, Krasheninnikov ME, et al. The method of production the preparation of the human neurons for cytotransfusion. The Russian Federation Patent No. 2146932 RU.

[15] Bryukhovetskiy AS, Yarygin VN, Chekhonin VP, et al. Implantation of the biodegradable polymer matrix SpheroGel and embryonic stem cells under experimental complete spinal injury. Presented at the 7th International Congress of the Cell Transplant Society. Boston, USA, 2004.

[16] Bryukhovetskiy AS, Polumbo O. Preparation of autologous non-hematopoetic progenitor stem cells, method of preparation and use thereof International Application № PCT/EP 2006/009008 filed September 15, 2006.

[17] Tupitsin NN, Yaryghin VN, Grivtsova LY, et al. Immunophenotypic peculiarities of mobilized stem (CD34+) cells in blood from patients with severe spinal cord injury. *J. of Biological Regulators and Homeostatic Agents*. 2006, **20**(1–2): 36–40.

[18] Bryukhovetskiy AS Myshkin OA, Avdeykin SN, et al. efficiency and safety assessment of 50 tissue engineering surgeries in spinal cord. Presented at the TERMIS-NA2010 Annual Conference, Orlando, USA, December 5–8, 2010.

[19] Bryukhovetskiy AS, Myshkin O, Avdeikin S, et al. Fifty surgeries in tissue engineering of spinal cord injury with equivalent of artificial nervous tissue in humans. Presented

at 2011 Translational Regenerative Medicine Forum, Washington, DC, USA, April 6–8, 2011.

[20] Bryukhovetskiy AS, Yevseev N, Avdeykin S, et al. Comparative analysis of long-term outcomes of various stem cell therapies of spinal cord injury in humans. Presented at IANR V & 9th GCNN Conference with ISCITT Symposium, Xi'an, China, 4–7 May, 2012.

[21] Bryukhovetskiy AS, Bryukhovetskiy IS. Remote multi-wave radioneuroengineering: An innovative technology for non-contact radio restoration of damaged nervous tissue of the human brain and spinal cord. *Translational Neuroscience and Clinics* 2015, **1**(1): 1–29.

[22] Bryukhovetskiy AS, Bryukhovetskiy IS. Effectiveness of repeated transplantations of hematopoietic stem cells in spinal cord injury. *World J. Transplant*. 2015, **5**(3): 110–128

[23] Frolov AA, Bryukhovetskiy AS. Effect of hematopoietic autologous stem cell transplantation to the chronically injured human spinal cord evaluated by motor and somatosensory evoked potentials methods. *Cell Transplant*. 2012, **21**(Suppl 1): S49–55.