

Effect of implantation of a PHPMA hydrogel containing human mesenchymal stromal cells of different origins on hindlimb locomotor function recovery in rats with spinal cord injury



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ABSTRACT

Spinal cord injury is a relatively common type of trauma under combat conditions and, in addition to an increased risk of mortality, in most cases results in impairments of motor and other functions. Restorative treatment of this injury remains one of the most challenging problems in medicine, and its solution is associated with the use of bioengineered implants in combination with stem cells capable of promoting regenerative axonal growth into the denervated regions of the spinal cord.

OBJECTIVE. To determine the effect of implantation of a PHPMA hydrogel populated with human mesenchymal stem/stromal cells (MSCs) of different origins on hindlimb locomotor function recovery in rats after experimental spinal cord injury.

MATERIALS AND METHODS. Spinal cord injury was modelled in 70 adult male outbred albino rats aged 3-4 months by left-sided excision of a fragment corresponding to one half of the spinal cord cross-section at the thoracolumbar level. For implantation into the injury site, PHPMA hydrogel (HG) or hydrogel populated with MSCs derived from the wall of the human umbilical artery (MSC-UA) or from derma (MSC-Dr) were used. The control group consisted of animals with spinal cord injury model that received no treatment. Locomotor activity and spasticity in the paretic limb were assessed on days 7 and 14 and subsequently monthly for up to 4 months post-injury using the Basso-Beattie-Bresnahan (BBB) scale and the Ashworth scale, respectively.

RESULTS. Four months after injury, the highest motor function scores were observed in the MSC-UA group, the lowest – in the MSC-Dr group, and intermediate values – in the other groups. Significant differences were detected between the control and MSC-UA groups and between the MSC-UA and MSC-Dr groups ($p < 0.05$). A significant increase in motor function score in the control group was observed during the first 2 months after injury, in the HG group – during the first month, and in the MSC-Dr and MSC-UA groups – throughout the entire 4-month observation period.

Four months after injury modelling, the highest spasticity scores were observed in the MSC-Dr group, whereas the lowest were observed in the control and MSC-UA groups; statistically significant differences were found only between the MSC-UA and MSC-Dr groups ($p < 0.02$). In addition, a significant negative correlation between individual motor function and spasticity scores was identified in all groups, with the strongest correlation in the MSC-Dr and SCI groups.

CONCLUSION. Implantation of PHPMA hydrogel populated with human umbilical artery-derived MSCs into the spinal cord defect in rats promotes improved recovery of locomotor function in the paretic hindlimb at 4 months of follow-up. However, it does not exert a significant effect on spasticity. The use of cell-free hydrogel or hydrogel seeded with dermal MSCs did not result in an improvement in locomotor activity or spasticity of the paretic hindlimb in rats.

KEY WORDS: spinal cord injury; PHPMA hydrogel; mesenchymal stem/stromal cells; hindlimb locomotor function; spasticity

Traumatic spinal cord injury (SCI) remains a critical benchmark of the capabilities of contemporary biomedical science and clinical practice. Although the absolute number of affected patients is relatively small, SCI is characterized by substantial prevalence and high socioeconomic burden [1-3]. Moreover, SCI profoundly impairs quality of life and may reduce life expectancy due to a wide spectrum of complications that can lead to premature mortality [4-5]. In the structure of combat-related trauma, the proportion of SCI is even more pronounced and may reach up to 8 % [6].

Currently, evidence-based therapeutic interventions for SCI include early spinal cord decompression with reconstruction and stabilization of the injured spine within the first 24 hours, prevention of thrombotic complications, and possibly maintenance of elevated mean arterial pressure to ensure adequate hemoperfusion across the injury site [7, 8]. Other therapeutic approaches for SCI have not yet demonstrated clinically verified efficacy [8-13].

Restorative strategies for SCI encompass various transplantation-based interventions, physical rehabilitation techniques, implantable and non-invasive modalities of chronic electrical or magnetic brain stimulation, as well as brain-machine interface technologies [9, 11, 13, 14]. The most promising direction in the development of restorative treatment for SCI is considered to be the integration of these multimodal approaches into combined therapeutic strategies [9, 15, 16].

Stem cell populations investigated in this field include neural stem cells (NSCs) and mesenchymal stem/stromal cells (MSCs) [9, 11]. Both autologous and allogeneic sources of these cells are being explored, including induced pluripotent stem cells (iPSCs) [9, 11]. Despite the considerable number of clinical trials assessing the efficacy of cell transplantation in SCI, none of the proposed therapeutic algorithms has accumulated sufficient safety data, and the overall clinical efficacy of this approach remains modest [8, 9, 11, 16-18].

One of the promising directions in tissue engineering involves the use of synthetic scaffolds, particularly hydrogels. Among these, a macroporous hydrogel based on poly(N-[2-hydroxypropyl]-methacrylamide (PHPMA) hydrogel has been extensively investigated in models of laceration-type SCI [19-27]. However, the spectrum of cell types whose transplantation in combination with this scaffold provides the most beneficial effect on post-SCI regenerative processes remains incomplete.

A distinct clinical challenge is the management of three major late-stage neurological complications of SCI – spasticity, chronic pain, and urinary dysfunction [28-31]. At least spasticity and chronic pain may share common pathophysiological mechanisms [32-34]. The development of chronic pain following SCI is directly associated with the activation and persistence of inflammatory processes [30, 35], and neural transplantation procedures may initiate or directly modulate these responses [36]. Conversely, MSCs exert immunotropic and anti-inflammatory effects [37]. Therefore, elucidating the impact of cell transplantation on the course of chronic SCI-related disorders remains highly relevant. Moreover, data regarding the efficacy of MSCs derived from human umbilical arteries and skin dermis in SCI are currently lacking.

Accordingly, **THE AIM** of this study was to determine the effect of implantation of a PHPMA hydrogel pre-seeded with human umbilical artery-derived MSCs or skin dermis-derived MSCs on motor function recovery and spasticity in the paretic limb following unilateral laceration-type SCI in rats.

MATERIALS AND METHODS

Spinal cord injury modeling. The study was performed on adult outbred male albino rats (250-300 g, 3-4 months old) obtained from the vivarium of the State Institution "Romodanov Neurosurgery Institute of the National Academy of Medical Sciences of Ukraine". Animals were housed under a natural light-dark cycle at room temperature with ad libitum access to balanced pelleted feed and water. A unilateral left-sided hemisection of the spinal cord at the lower thoracic-upper lumbar level was used as the SCI model [38].

Four experimental groups were formed:

1. SCI (spinal cord injury) – SCI modeling only (n = 22; n = 19 completed the observation period);
2. HG (hydrogel) – SCI modeling + immediate implantation of PHPMA hydrogel (n = 18; n = 15 completed the observation period);
3. MSC-UA (mesenchymal stem/stromal cells from the umbilical artery wall) – SCI modeling + immediate implantation of PHPMA hydrogel containing human umbilical artery wall-derived MSCs (n = 25; n = 20 completed the observation period);
4. MSC-Dr (mesenchymal stem/stromal cells of dermal origin) – SCI modeling + immediate implantation of PHPMA hydrogel containing adult human dermis-derived MSCs (n = 20; n = 16 completed the observation period).

The study was conducted in accordance with the principles of bioethics and humane treatment of animals. The experimental protocol was approved by the Bioethics Commission and Ethics Committee for Scientific Research of Bogomolets National Medical University (Protocol No. 172, dated 22 June 2023).

The SCI model used in this study has been described in detail in our previous publication [38]. Briefly, surgical procedures were performed under general anesthesia induced by intraperitoneal administration of 15 mg/kg xylazine (*Xyla, Interchemie werken De Adelaar BV*, the Netherlands) and 70 mg/kg ketamine (*Farmak, Ukraine*). Following a linear skin incision, a limited left-sided laminectomy was performed at the level of approximately Th11-Th12 vertebrae corresponding to spinal cord segments ~Th13-L1. Without separating the dura mater or spinal roots, the spinal cord was punctured ventrally with an insulin needle at two points (~1 mm apart) along the left border of the posterior median artery. A longitudinal paramedian incision between the puncture sites was then made using ophthalmic scissors. The tissue of the left hemicord was transected at the rostral and caudal margins of the incision and removed from the wound using microsurgical forceps of varying geometry under magnification provided by a surgical microscope.

The resulting spinal cord defect was left unfilled (SCI group), filled with a fragment of PHPMA hydrogel (HG group), or filled with a fragment of PHPMA hydrogel containing MSCs derived from the human umbilical artery wall or adult human dermis (MSC-UA and MSC-Dr groups, respectively). In all animals, the bone defect was covered with a fragment of subcutaneous fascia, and the soft tissues and skin were closed in two layers using interrupted sutures. To prevent infectious and inflammatory complications, 0.5 million IU/kg Bicillin-5 (*Kyivmedpreparat, Ukraine*) was administered subcutaneously into the posterior cervical region and 5 mg/kg dexamethasone solution (*KRKA, Slovenia*) was administered intraperitoneally. Postoperatively, animals were maintained for 2-4 hours in a room with elevated ambient temperature (30 °C) and subsequently housed under standard conditions (see above) in medium-sized cages in groups of several animals [38].

Assessment of motor activity and spasticity in the paretic limb. Following successful induction of SCI, animals exhibited motor deficits of the ipsilateral hindlimb in the form of spastic paresis of varying severity. Motor function and spasticity of the paretic limb were evaluated on days 7 and 14 and monthly thereafter following injury using the Basso-Beattie-Bresnahan (BBB) locomotor rating scale and the Ashworth scale, respectively, in our technical modifications [38], without prior training of the animals.

The BBB scale enables assessment of hindlimb locomotor function during spontaneous locomotion in an open field. It includes evaluation of joint movements in three key joints (0-9 points), the ability and degree of weight support by the paretic limb (9-11 points), interlimb coordination (12-14 points), fine motor control of the paw muscles (14-20 points), maintenance of tail position above the plane of locomotion (19-20 points), and trunk stability (20-21 points) [38].

Although the Ashworth scale is designed to detect the principal dynamic manifestation of spasticity – the so-called “catch” phenomenon, defined as a marked increase in resistance to rapid passive joint

movement – in rats it primarily reflects resistance during passive flexion-extension movements of the joints in the paretic limb. Accordingly, the scale grades spasticity from the absence of resistance to passive movement (0 points) to severe restriction of passive joint mobility, including pronounced contracture and, in some cases, ankylosis [38]. The total follow-up period was 4 months after SCI induction.

Compliance with inclusion criteria. All animals in the experimental groups demonstrated a paretic hindlimb motor recovery of < 9 points on the BBB scale at the first assessment time point (day 7 after SCI induction). Throughout the experiment, animals did not exhibit persistent motor deficits of the contralateral hindlimb (≤ 14 points on the BBB scale), signs of stable peripheral paresis indicative of lower motor neuron damage at the L3-L6 segments, or evidence of trophic disturbances or traumatic injury of the paretic limb. Cases of abdominal wall muscle paresis were not specifically recorded [38].

Isolation, culture, immunophenotyping, and directed differentiation of MSCs. MSC-UA were isolated from human umbilical arteries ($n = 2$). Umbilical cord tissue samples obtained during physiological deliveries from women aged 29-37 years at 38-41 weeks of gestation (one male and one female neonate). MSC-Dr were isolated from human skin samples obtained by punch biopsy ($n = 2$) from donors aged 27-36 years, one male and one female. All tissue samples were obtained according to informed consent.

Cells were isolated under sterile conditions using an enzymatic digestion method (0.1 % collagenase (Gibco, USA) and 0.1 % hyaluronidase (Sigma, Germany), 45-50 min at 37 °C). Primary cultures and subsequent passages were performed at 80-85 % monolayer confluence. Cells were detached using 0.025 % trypsin/EDTA solution (Capricorn, Germany) and replated into new flasks at a density of $3-4 \times 10^3$ cells/cm². Cells were cultured in DMEM/F12 medium supplemented with 5 % FBS, 1 % L-glutamin, 0.2 % antibiotic/antimycotic solution (all – Capricorn, Germany), and 1 ng/mL bFGF (Sigma, Germany). The culture medium was changed every 48 hours.

The percentage of viable and dead cells at passage 2 was determined using a BD FACSAria laser flow cytometer-cell sorter based on 7-AAD (BD, USA) uptake by membrane-compromised cells. Expression levels of surface markers CD73, CD90, CD105, CD34, CD45, and HLA-DR (all – BD Pharmingen, USA) were also assessed using the BD FACSAria cell sorter. Detailed protocol descriptions have been published previously [25]. Osteogenic and adipogenic differentiation of both stem cell types was induced using standard protocols for 21 and 14 days, respectively, as described earlier [25].

Immunocytochemical characterization of stem cells. MSC-UA and MSC-Dr cultures grown on glass coverslips were fixed in 4 % formaldehyde and subsequently treated with a blocking/permeabilization solution containing 0.3 % Triton X-100 (Sigma, USA) and 0.5 % bovine serum albumin (Sigma, USA) to enhance antibody penetration and minimize nonspecific binding.

Samples were incubated for 24 hours with a mixture of primary antibodies. To identify MSCs and assess their proliferative activity, double immunocytochemical staining was performed using chicken anti-vimentin antibodies (1:2500, Dako, USA) and rabbit anti-Ki-67 antibodies (1:200, Thermo Fisher Scientific, USA). Cell nuclei were counterstained with the fluorescent dye Hoechst 33342 (1:5000, Sigma, USA). After washing in 0.1 M phosphate buffer, specimens were incubated for 1.5 hours with a mixture of secondary antibodies: anti-chicken Alexa Fluor 488-conjugated antibodies (1:1000, Invitrogen, USA) and anti-rabbit Alexa Fluor 555-conjugated antibodies (1:1000, Invitrogen, USA).

Cell cultures were mounted in Fluoromount™ Aqueous Mounting Medium (Sigma, USA) and analyzed using a FluoView™ FV1000 confocal laser scanning microscope (Olympus Inc., Japan) equipped with a digital camera interfaced with a computer for image acquisition and analysis.

Preparation of PHPMA hydrogel and cell populating. The PHPMA hydrogel is a polymeric material synthesized from N-(2-hydroxypropyl) methacrylamide (Aqua Gel Technologies, Canada) under conditions

of heterophase separation by radical polymerization in a porogen-containing medium with the formation of divinyl cross-links [24-26]. After removal of residual by-products and high-temperature sterilization, macroscopic PHPMA hydrogel fragments (3-4 cm in length and approximately 1 cm in diameter) were transported in distilled water in sealed containers and autoclaved immediately prior to use.

Both stem cell types were populated by direct injection of a cell suspension (50 μ L, 3×10^4 cells) into partially dehydrated (air-dried under laminar airflow conditions) PHPMA hydrogel fragments (12 mm², molded into plastic forms with tapered ends). Hydrogel fragments containing the introduced cells were placed into 4-well culture plates (Nunc, Denmark) filled with culture medium. The constructs (PHPMA + MSC-UA and PHPMA + MSC-Dr) were further cultured for 7 days, with medium changes every 48 hours. The culture medium was identical to that described above.

Statistical analysis. Statistical analysis was performed using the EZR software package (R-statistics), which is freely available, on a personal computer. Mean locomotor function and spasticity score values in the compared samples were presented as median (Q1; Q3), since the distribution of values in the analyzed samples deviated from normality.

In experimental animals that completed the observation period, differences in locomotor function and spasticity values across observation time points within each group were assessed using repeated-measures ANOVA (rANOVA) or the Friedman test; Bonferroni correction was applied for multiple comparisons in both cases. Additionally, to complement and clarify the statistical findings, paired comparisons were performed using the paired Student's t-test (for normally distributed data) or the Wilcoxon signed-rank test (for non-normal distributions).

To determine the significance of intergroup differences in locomotor function and spasticity at each observation time point, normality was assessed using the Shapiro-Wilk test. In cases of normal distribution, homogeneity of variances was evaluated using Bartlett's test. Intergroup comparisons were then conducted using one-way ANOVA with Tukey's post hoc test (for normally distributed data) or the Kruskal-Wallis test with the Steel-Dwass post hoc test (when distributions deviated from normality or variances were heterogeneous). To further substantiate intergroup comparisons, pairwise analyses of independent samples were performed using the unpaired Student's t-test (for normal distributions) or the Wilcoxon-Mann-Whitney test (for non-normal distributions).

Correlations between locomotor function and spasticity were assessed for animals that completed the observation period (see above) using Pearson's correlation coefficient (when at least one variable followed a normal distribution) or Spearman's rank correlation test (when both variables deviated from normality). In all analyses, results were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Characterization of stem cells used for transplantation. From the first passage onward (including passages 2 and 3), both MSC phenotypes displayed a fibroblast-like morphology, strong adherence to plastic, and a population of proliferatively active cells as indicated by Ki-67 staining (Fig. 1).

Viability of MSC-UA and MSC-Dr, assessed by a BD FACSAria cell sorter, was 93.5 ± 3.3 % ($n = 2$) and 96.3 ± 2.6 % ($n = 2$), respectively. Immunophenotyping of passage 2 cultures (MSC-UA, $n = 2$; MSC-Dr, $n = 2$) revealed characteristic expression profiles of surface markers. MSC-UA exhibited high expression of CD73 (99.4 ± 0.5 %), CD90 (98.8 ± 1.2 %), and CD105 (98.0 ± 0.7 %), whereas MSC-Dr expressed CD73 (99.9 ± 0.0 %), CD90 (99.5 ± 0.1 %), and CD105 (99.1 ± 0.6 %), consistent with the mesenchymal phenotype of these cells. Expression of CD34, CD45, and HLA-DR was below 1 % in both cell types.

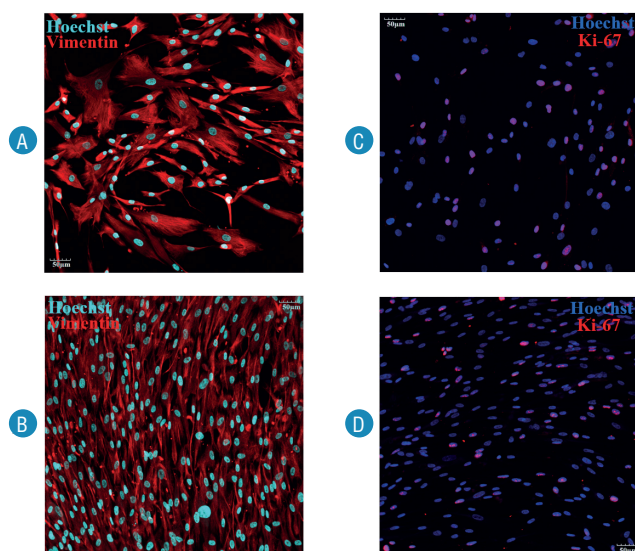


Fig. 1. Microphotographs of MSC-Dr (A, C) and MSC-UA (B, D) cultures, passage 2; double immunocytochemical staining. A-B: Vimentin – red, Hoechst – cyan. C-D: Ki-67 – red, Hoechst – blue. Laser scanning confocal microscopy, scale bar – 50 µm.

Following directed adipogenic differentiation for 14 days, Oil Red O staining demonstrated abundant intracellular lipid droplets, indicating successful differentiation of MSCs into adipocytes. Directed osteogenic differentiation for 21 days, assessed via BCIP/NBT substrate reaction (*Sigma*, USA), revealed strong alkaline phosphatase activity as a marker of osteoinduction, confirming differentiation of both MSC-UA and MSC-Dr into osteocytes. Alizarin Red staining further demonstrated mineral deposition in the extracellular matrix, supporting the osteogenic differentiation capacity of both cell types.

Overall, MSC-UA and MSC-Dr cultures met the minimal criteria for multipotent stem cells, exhibiting typical fibroblast-like morphology, adherence to plastic, proliferative potential, characteristic immunophenotype, and the ability to differentiate into adipogenic and osteogenic lineages.

Dynamics of locomotor function and spasticity of the paretic hindlimb. At day 7 post-injury, locomotor function values in all experimental groups deviated from a normal distribution ($p < 0.01$; Shapiro-Wilk test). Median values were as follows: SCI group – 1 (1;1) BBB points, HG – 3 (1;6), MSC-Dr – 1 (1;2), and MSC-UA – 2 (1;6) (**Fig. 2**). Locomotor function values at this time point differed significantly for pairwise comparisons between SCI and HG, and SCI and MSC-UA ($p < 0.05$; Steel-Dwass post hoc test) or between SCI and HG, SCI and MSC-UA, HG and MSC-Dr, MSC-UA and MSC-Dr ($p < 0.03$; Wilcoxon-Mann-Whitney test) (**Fig. 2**).

The greatest increase in locomotor function across all groups occurred during the following week. By day 14 post-injury, median values were: SCI – 4.5 (4; 6) BBB points, HG – 7 (3; 8), MSC-Dr – 2 (2; 6), and MSC-UA – 7 (6; 8). At this time, locomotor function differed significantly for comparisons between MSC-Dr and HG, and MSC-Dr and MSC-UA ($p < 0.05$; Steel-Dwass post hoc test) or between SCI and MSC-UA, HG and MSC-Dr, MSC-UA and MSC-Dr ($p < 0.03$; Wilcoxon-Mann-Whitney test). Locomotor function score distributions were non-normal in HG, MSC-Dr, and MSC-UA groups ($p < 0.01$; Shapiro-Wilk test).

At 4 months post-injury, locomotor function score distributions deviated from normal only in MSC-UA and MSC-Dr groups ($p < 0.01$; Shapiro-Wilk test). Median values were: SCI – 7 (6.5; 8), HG – 9 (5; 11), MSC-Dr – 7 (3; 9), MSC-UA – 9 (8; 10) BBB points (**Fig. 2**). Locomotor function values differed significantly only for the SCI vs MSC-UA comparison ($p < 0.03$; Steel-Dwass post hoc test) or for SCI vs MSC-UA and MSC-UA vs MSC-Dr ($p < 0.05$; Wilcoxon-Mann-Whitney test) (**Fig. 2**).

Overall, the highest locomotor function values were observed in the MSC-UA group and the lowest in the MSC-Dr group, indicating a positive effect of MSC-UA transplantation on recovery of motor function in the ipsilateral hindlimb following spinal cord injury.

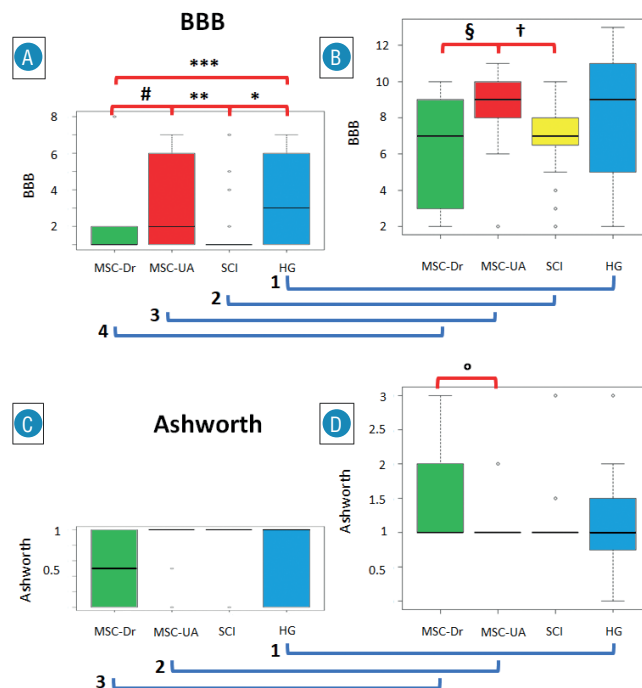


Fig. 2. Median values of locomotor function (BBB – panels A and B) and spasticity (Ashworth – panels C and D) are shown for four experimental groups at 7 days (A and C) and 4 months (B and D) post-injury. Horizontal ticks within vertical boxes indicate median values; shaded portions of the boxes represent the first and third quartiles (below and above the median, respectively); points inside the boxes indicate mean values; vertical box extents represent standard deviations; whiskers above and below the boxes show the data range beyond the first and third quartiles.

Notes. Statistically significant differences in pairwise comparisons are indicated as follows:

Panel A (motor function values at 7 days post-injury):

* – SCI vs HG, $p < 0.05$ (Steel-Dwass post hoc), $p < 0.01$ (Wilcoxon-Mann-Whitney);

** – SCI vs MSC-UA, $p < 0.05$ (Steel-Dwass), $p < 0.01$ (Wilcoxon-Mann-Whitney);

*** – HG vs MSC-Dr, $p < 0.05$ (Wilcoxon-Mann-Whitney);

– MSC-UA vs MSC-Dr, $p < 0.05$ (Wilcoxon-Mann-Whitney).

Panel B (motor function values at 4 months post-injury):

† – SCI vs MSC-UA, $p < 0.05$ (Steel-Dwass), $p < 0.01$ (Wilcoxon-Mann-Whitney);

§ – MSC-UA vs MSC-Dr, $p < 0.05$ (Wilcoxon-Mann-Whitney).

Panels A-B (motor function comparison within each group across two time points, performed for animals that completed the observation period: SCI – $n = 19$, HG – $n = 15$, MSC-UA – $n = 20$, MSC-Dr – $n = 16$):

1 – HG, $p < 0.05$ (Friedman with Bonferroni), $p < 0.001$ (Wilcoxon);

2 – SCI, $p < 0.01$ (Friedman with Bonferroni), $p < 0.001$ (Wilcoxon);

3 – MSC-UA, $p < 0.01$ (Friedman with Bonferroni), $p < 0.001$ (Wilcoxon);

4 – MSC-Dr, $p < 0.05$ (Friedman with Bonferroni), $p < 0.001$ (Wilcoxon).

Panel D (spasticity values at 4 months post-injury):

° – MSC-UA vs MSC-Dr, $p < 0.05$ (Wilcoxon-Mann-Whitney)

Panels C-D (spasticity comparison within each group across two time points):

1 – HG, $p < 0.05$ (Friedman with Bonferroni), $p < 0.05$ (Wilcoxon);

2 – MSC-UA, $p < 0.05$ (Friedman with Bonferroni), $p < 0.01$ (Wilcoxon);

3 – MSC-Dr, $p < 0.01$ (Friedman with Bonferroni), $p < 0.01$ (Wilcoxon).

Over the entire observation period, locomotor function in the SCI, HG, and MSC-UA groups changed most intensively during the first two weeks. At 2 weeks post-injury, the median locomotor values in the SCI group reached 64 % of the values observed at 4 months, whereas in the HG and MSC-UA groups, they reached 78 %. In contrast, in the MSC-Dr group, this value was only 29 %.

In the SCI group, locomotor values at 1 week were significantly lower than at subsequent time points ($p < 0.02$, Friedman test with Bonferroni correction), and showed significant increases between the first and second weeks, between the second week and the first month, and during the second month of observation ($p < 0.05$, Wilcoxon test; $p < 0.05$, Student's *t*-test).

In the HG group, a significant difference from locomotor function at 1 week post-injury was observed only for locomotor function measured at 1-4 months ($p < 0.04$, Friedman test with Bonferroni correction), while according to other analyses, locomotor function increased significantly only during the first month of observation ($p < 0.05$, Wilcoxon test).

In the MSC-Dr group, locomotor values at 1 week were significantly lower than at all later time points ($p < 0.02$, Friedman test with Bonferroni correction), but increased significantly over the first 2 months and at 4 months post-injury ($p < 0.05$, Wilcoxon test).

In the MSC-UA group, locomotor values increased significantly during the second week and the second month of observation ($p < 0.02$, Friedman test with Bonferroni correction), and, according to other analyses, over the first 2 months and at 4 months post-injury ($p < 0.02$, Wilcoxon test). In other words, significant locomotor function recovery occurred over 2 months in the SCI group, 1 month in the HG group, and 4 months in the MSC-UA group. These data indicate distinct dynamics of locomotor function improvement in each group.

The distribution of spasticity scores in all experimental groups at 7 days post-injury deviated from normality ($p < 0.01$, Shapiro-Wilk test). At this time point, median spasticity score values were as follows: SCI – 1 (1; 1) Ashworth, HG – 1 (0; 1), MSC-Dr – 0.5 (0; 1), and MSC-UA – 1 (1; 1) Ashworth (Fig. 2). There were no significant differences in spasticity score between the experimental groups at this time point ($p > 0.05$, Steel-Dwass test for post hoc comparisons; Wilcoxon-Mann-Whitney for pairwise comparisons). Throughout the observation period, spasticity score values increased slowly and irregularly across all experimental groups, reflecting gradual development of spasticity in the paretic limb post-injury.

At 1 month post-injury, the distribution of spasticity scores in all experimental groups deviated from normality ($p < 0.01$, Shapiro-Wilk test), with median spasticity score values of 1 (1; 1) Ashworth in the SCI, MSC-Dr, and MSC-UA groups, and 0.75 (0; 1) Ashworth in the HG group (Fig. 1). The spasticity values in the HG group at this time point differed significantly from those in the other three groups ($p < 0.04$, Steel-Dwass test for post hoc comparisons; $p < 0.01$, Wilcoxon-Mann-Whitney test for pairwise comparisons).

At 2 months post-injury, spasticity score distributions in all experimental groups again deviated from normality ($p < 0.01$, Shapiro-Wilk test). Median spasticity values were 1 (1; 1) Ashworth in the SCI and MSC-UA groups, 1 (0.625; 1) in the HG group, and 1 (1; 2) in the MSC-Dr group. At this time point, spasticity values did not differ significantly between groups ($p > 0.05$, Steel-Dwass test), although pairwise comparisons indicated a difference for the HG group relative to the other three groups ($p < 0.04$, Wilcoxon-Mann-Whitney test).

At 4 months post-injury, the spasticity score distribution was normal only in the HG group ($p > 0.05$, Shapiro-Wilk test). Median spasticity score values were 1 (1; 1) Ashworth in the SCI and MSC-UA groups, 1 (0.75; 1.5) in the HG group, and 1 (1; 2) in the MSC-Dr group (Fig. 2). At this time point, spasticity values did not differ significantly between groups ($p > 0.05$, Steel-Dwass test), except for a significant difference between MSC-UA and MSC-Dr in pairwise comparison ($p < 0.02$, Wilcoxon-Mann-Whitney test) (Fig. 2). Overall, the highest spasticity

values were observed in the MSC-Dr group, and the lowest in the SCI and MSC-UA groups. These data indicate that the presence of MSC-Dr in the hydrogel does not affect spasticity, whereas MSC-UA is associated with a dynamic reduction in PS.

Over the course of the entire experiment, a gradual increase in spasticity scores was observed in the HG and MSC-Dr groups, whereas the SCI and MSC-UA groups showed a rapid increase during the first month, followed by no significant changes over the subsequent three months. Overall, statistical analysis confirmed these observations. In the SCI group, significant changes in spasticity score were detected only during the second week of the experiment ($p < 0.001$; Friedman test with Bonferroni correction) or were not observed at all for all pairwise comparisons ($p > 0.05$; Wilcoxon test). In the HG group, a significant increase in spasticity score relative to 1-week values was observed only at 3 months post-injury ($p < 0.03$, Wilcoxon test; $p < 0.03$, Friedman test with Bonferroni correction). In the MSC-Dr group, spasticity score increased significantly over the first two months ($p < 0.03$, Wilcoxon test; $p < 0.03$, Friedman test with Bonferroni correction), while in the MSC-UA group, spasticity increased significantly only during the first month after injury ($p < 0.02$, Wilcoxon test; $p < 0.02$, Friedman test with Bonferroni correction). These results indicate that the presence of MSC-UA in the hydrogel promotes motor activity recovery in rats after SCI, whereas MSC-Dr does not exert such an effect.

As previously noted, at 4 months post-injury, the highest locomotor function scores were observed in the MSC-UA group and the lowest in the MSC-Dr group, with the other two groups showing intermediate values. Conversely, the highest spasticity values were observed in the MSC-Dr group and the lowest in the SCI and MSC-UA groups. This finding is consistent with the concept that reduced voluntary muscle control after SCI – that is, increased central paresis – is associated with higher spasticity, reflecting involuntary reflex muscle activity. Correlation analysis of the data, performed for animals that completed the observation period supported this notion. Specifically, analysis of individual locomotor function/spasticity scores pairs across all groups at each time point revealed a weak negative correlation at 1 month ($r_s = -0.29$, $p < 0.02$) and moderate negative correlations at 2, 3, and 4 months ($r_s = -0.54$, $p < 0.01$; $r_s = -0.62$, $p < 0.001$; $r_s = -0.66$, $p < 0.001$) (Fig. 3). However, at 7 days post-injury, the MSC-UA group exhibited a moderate positive correlation between individual locomotor and spasticity values ($r_s = +0.59$, $p < 0.01$), whereas the SCI group showed a moderate negative correlation ($r_s = -0.53$, $p < 0.05$).

At 2 months post-injury, a strong negative correlation between individual locomotor and spasticity values was observed in the MSC-Dr group ($r_s = -0.76$, $p < 0.001$) (Fig. 3), while the SCI group showed a moderate negative correlation ($r_s = -0.68$, $p < 0.01$). At 3 months post-injury, the MSC-Dr group exhibited an even stronger negative correlation ($r_s = -0.82$, $p < 0.001$), whereas the SCI group maintained a moderate negative correlation ($r_s = -0.63$, $p < 0.01$). Similarly, at 4 months post-injury, the MSC-Dr group showed a very strong negative correlation between individual locomotor and spasticity values ($r_s = -0.86$, $p < 0.001$) (Fig. 3), while the SCI group demonstrated a strong negative correlation ($r_p = -0.73$, 95 % CI -0.89 ... -0.41, $p < 0.001$). Interestingly, at this same time point, both the MSC-UA and HG groups displayed moderate negative correlations ($r_s = -0.49$, $p < 0.05$ and $r_p = -0.57$, 95 % CI -0.84 ... -0.08, $p < 0.05$, respectively).

Tissue neuroengineering is an actively researched biomedical field aimed at restoring lost nervous system functions by recreating its microstructure [17, 39-41]. In cases of spinal cord injury (SCI), when the lesion is located outside the motor innervation zones of the limbs, the primary goal of regenerative treatment is the restoration of supraspinal innervation of motor neurons – for example, by promoting the growth of injured long-projection axons through the post-traumatic reorganization zone. Neuroengineering tools that can address this challenge currently include transplantation- and molecular-genetic-based approaches aimed at stimulating axon growth through the injury site,

promoting their myelination, and neutralizing factors that inhibit axonal regeneration [9, 10, 11, 15-17]. Considering that voluntary stepping synergies in mammals correlate with the preservation of only a small fraction of spinal white matter tracts, achieving significant positive outcomes in SCI treatment using such bioengineered approaches is realistic [42].

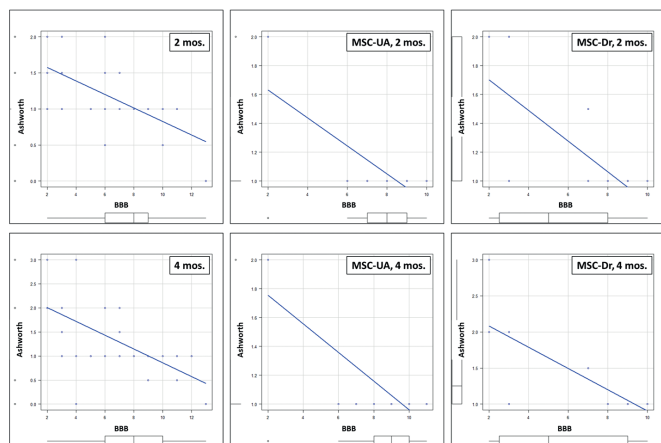


Fig. 3. Correlation between BBB (locomotor function) and Ashworth (spasticity) scores across the samples of animals that completed the observation period. Top row, left to right: all individual locomotor/spasticity scores pairs at 2 months – moderate negative correlation: $r_s = -0.54$ ($p < 0.01$); MSC-UA group at 2 months – no significant correlation: $r_s = -0.39$ ($p > 0.05$); MSC-Dr group at 2 months – strong negative correlation: $r_s = -0.76$ ($p < 0.001$). Bottom row, left to right: all individual locomotor/spasticity scores pairs at 4 months – moderate negative correlation: $r_s = -0.66$ ($p < 0.001$); MSC-UA group at 4 months – moderate negative correlation: $r_s = -0.49$ ($p < 0.05$); MSC-Dr group at 4 months – strong negative correlation: $r_s = -0.86$ ($p < 0.001$).

Currently, restorative neurosurgery is at the stage of testing amorphous porous matrices loaded with one or multiple cell types, as well as the development of spatially organized matrices, including those combined with immature cells [17, 43]. Since the most common form of SCI in humans is contusion and/or compression, transplantation of intact volumetric biocomposite structures may have clinical application for obvious reasons [7, 44-46]. However, this will only be justified if their efficacy supports the removal of damaged spinal cord tissue or already formed scar tissue, without disregarding nerve fibers that may have survived in the injury zone or grown de novo.

To date, the use of implantable materials – even in combination with other spinal cord regeneration-enhancing strategies – has been shown in experimental studies to improve motor function recovery after SCI by 25.3 % [47]. The overall efficacy of all experimental regenerative treatments for SCI averages an 18 % improvement in motor function according to the BBB scale [48]. Our results fall within this range of efficacy, significantly enhancing the recovery process after SCI in adult rats by approximately 10 % when an PHPMA hydrogel associated with human umbilical artery wall MSCs was implanted into the injury site, compared with implantation of RHPMA hydrogel alone. It should be noted that, at present, none of the clinical bioengineering algorithms for SCI treatment have generated sufficient data regarding efficacy and safety, and overall, the effectiveness of these methods remains suboptimal [8, 9, 11, 16-18]. However, a substantial number of potential adverse reactions associated with cell transplantation in SCI have been reported [16, 18].

Transplanted stem cells in spinal cord tissue likely undergo a phase of secretion of various bioactive substances and factors (the first week post-transplantation), followed by a differentiation phase (weeks 2-3) during which secretory function is lost, and then an immune elimination

phase [49]. The proposed mechanisms underlying the beneficial effects of MSCs include immunomodulatory, anti-inflammatory, pro-angiogenic, anti-apoptotic, and antioxidant effects mediated by numerous cytokines and growth factors. In addition, MSCs exert direct receptor-mediated effects on host cells, transfer mitochondria, produce extracellular microvesicles, possess the capacity for neural transdifferentiation – including differentiation into neuroglial cells – can fuse with recipient cells, and exhibit pro-neuroplastic effects [37, 50-56]. If MSCs derived from the umbilical artery wall have a specifically committed effect on vascular cells, their positive impact on SCI outcomes may be mediated by roles in restoring microcirculatory flow and the integrity of the spinal blood-brain barrier during the development of the early inflammatory process following SCI [57, 58].

Presumably, the peak activity of the intrinsic physiological plasticity process after SCI occurs during the first weeks of the post-injury period. However, when selecting the timing for a regenerative intervention, it is recommended to consider the dynamics of local inflammatory responses and secondary damage during the acute phase of injury [17, 59]. Therefore, in experimental settings, the optimal window for cell transplantation is considered to be between days 4 and 7 post-SCI [60, 61].

Despite the availability of various methods of treating spasticity after SCI with differing levels of efficacy, it is evident that restoring descending innervation of spinal motor neurons – particularly using bioengineering approaches – is the most logical strategy for simultaneously improving motor function and reducing spasticity after spinal cord injury [29, 62-66]. Our data generally support this assumption: the restoration of motor activity, i.e., voluntary innervation of paretic muscles, is accompanied by a reduction in homonymous manifestations of spasticity. However, the effect of cell transplantation on spasticity through modulation of the inflammatory process in the injured spinal cord remains unexplored.

One challenge in the clinical translation of experimental findings is the significant difference in size between commonly used small experimental mammals and humans, the inability to replicate all nuances of human SCI in experimental models, and the limitations of the current tools for assessing functional outcomes in small mammals [38, 44, 59, 67]. In particular, significant concerns exist regarding the use of the BBB scale to study unilateral motor deficits following the hemiexcision model implemented in our study, as well as the subjectivity of the Ashworth scale and the limited precision of electromyographic methods [38]. According to our observations, the limitations of the BBB scale are especially relevant for motor deficits corresponding to scores above 8 [38], which may reduce the reliability of the data obtained in this study and represents one of the factors limiting clinical translation and reproducibility [68]. On the other hand, considering the higher incidence of SCI in men [69], the results obtained in this study still hold significant practical relevance.

The results obtained in the SCI and HG groups, as noted, in adult animals using a hemiexcision spinal cord injury model differ from those observed in animals aged 1 month subjected to the same type of injury [24, 38, 70]. In young animals, excision of the lateral half fragment of the spinal cord, unlike hemisection in animals of the same age and origin, leads to a profound, non-recoverable deficit in motor function of the paretic limb, whereas immediate implantation of PHPMA hydrogel into the defect site significantly reduces this deficit by ~2.5 points on the BBB scale [24, 38, 70]. The lack of effect of PHPMA hydrogel in our adult animals is similar to its absence of influence on recovery after immediate implantation into the lateral hemisection site in young animals, although with lower BBB scores by ~2 points [24]. We suggest that these differences can be explained by age-dependent variations in the size of the axonal and dendritic apparatus of spinal interneurons, which mediate collateral pathways for descending inputs to motor neurons below the injury level, as well as by technical differences in model execution by different experimenters and variations in the lineage and housing conditions of experimental animals.

On the other hand, it should be noted that the number of studies on hydrogels, particularly PHPMA derivatives seeded with various cell types, including stem cells, remains very limited. This creates a knowledge gap regarding how synthetic matrices affect cellular proliferative status, phenotype, mitotic activity, and differentiation capacity under first-order contact conditions or during long-term survival within the matrix [25, 26, 71-73].

Currently, an alternative approach for restoring lost function after SCI is abiotic and bionic prosthetics, including “exoskeleton” technologies [14, 74-76]. However, these technologies have significant limita-

tions, such as unreliable electrode-tissue interfaces in neural prostheses or, for exoskeletons, the requirement for a minimum level of voluntary motor activity in paretic muscles and partial preservation of spasticity, i.e., retention of some descending spinal cord fibers [77]. Therefore, the development of effective biological strategies for regenerative treatment remains highly relevant, even in light of substantial progress in abiotic technologies for restoring spinal functions.

CONCLUSION

The obtained data overall demonstrate a significant positive effect of umbilical artery-derived MSC transplantation in association with PHPMA hydrogel on the course of laceration SCI. Specifically, implantation of PHPMA hydrogel seeded with MSC-UA into the hemisection site of the rat spinal cord improves recovery of motor function in the paretic limb over a 4-month observation period but does not significantly affect spasticity. In contrast, implantation of hydrogel without cells or seeded with MSC-Dr does not show a significant positive effect on locomotor activity or spasticity of the paretic limb.

REFERENCES:

- GBD 2016 Traumatic Brain Injury and Spinal Cord Injury Collaborators. Global, regional, and national burden of traumatic brain injury and spinal cord injury, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* 2019; 18(1):56-87. [https://doi.org/10.1016/S1474-4422\(18\)30415-0](https://doi.org/10.1016/S1474-4422(18)30415-0)
- Furlan JC, Gulasingham S, Craven BC. The Health Economics of the spinal cord injury or disease among veterans of war: A systematic review. *J Spinal Cord Med.* 2017; 40(6):649-664. <https://doi.org/10.1080/10790268.2017.1368267>
- Malekzadeh H, Golpayegani M, Ghodsi Z, Sadeghi-Naini M, Asgardoost M, Baigi V, et al. Direct Cost of Illness for Spinal Cord Injury: A Systematic Review. *Global Spine J.* 2022; 12(6):1267-1281. <https://doi.org/10.1177/21925682211031190>
- Cardile D, Calderone A, De Luca R, Corallo F, Quartarone A, Calabrò RS. The Quality of Life in Patients with Spinal Cord Injury: Assessment and Rehabilitation. *J Clin Med.* 2024; 13(6):1820. <https://doi.org/10.3390/jcm13061820>
- Johansson E, Koskinen E, Helminen M, Vainionpää A, Luoto TM. Mortality and causes of death of traumatic spinal cord injury in Finland. *Spinal Cord.* 2025; 63(1):24-30. <https://doi.org/10.1038/s41393-024-01047-9>
- Furlan JC, Gulasingham S, Craven BC. Epidemiology of War-Related Spinal Cord Injury Among Combatants: A Systematic Review. *Global Spine J.* 2019; 9(5):545-558. <https://doi.org/10.1177/2192568218776914>
- Eli I, Lerner DP, Ghogawala Z. Acute Traumatic Spinal Cord Injury. *Neurol Clin.* 2021; 39(2):471-488. <https://doi.org/10.1016/j.ncl.2021.02.004>
- Izzy S. Traumatic Spinal Cord Injury. *Continuum (Minneapolis Minn).* 2024; 30(1):53-72. <https://doi.org/10.1212/CON.0000000000001392>
- Flack JA, Sharma KD, Xie JY. Delving into the recent advancements of spinal cord injury treatment: a review of recent progress. *Neural Regen Res.* 2022; 17(2):283-291. <https://doi.org/10.4103/1673-5374.317961>
- de Almeida FM, Marques SA, Dos Santos ACR, Prins CA, Dos Santos Cardoso FS, Dos Santos Heringer L, et al. Molecular approaches for spinal cord injury treatment. *Neural Regen Res.* 2023; 18(1):23-30. <https://doi.org/10.4103/1673-5374.344830>
- Hu X, Xu W, Ren Y, Wang Z, He X, Huang R, et al. Spinal cord injury: molecular mechanisms and therapeutic interventions. *Signal Transduct Target Ther.* 2023; 8(1):245. <https://doi.org/10.1038/s41392-023-01477-6>
- Tang H, Gu Y, Jiang L, Zheng G, Pan Z, Jiang X. The role of immune cells and associated immunological factors in the immune response to spinal cord injury. *Front Immunol.* 2023; 13:1070540. <https://doi.org/10.3389/fimmu.2022.1070540>
- Yari D, Saberi A, Salmasi Z, Ghoreishi SA, Etemad L, Movaffagh J, et al. Recent Advances in the Treatment of Spinal Cord Injury. *Arch Bone Jt Surg.* 2024; 12(6):380-399. <https://doi.org/10.22038/ABJS.2023.73944.3424>
- He Y, Xu Y, Hai M, Feng Y, Liu P, Chen Z, et al. Exoskeleton-Assisted Rehabilitation and Neuroplasticity in Spinal Cord Injury. *World Neurosurg.* 2024; 185:45-54. <https://doi.org/10.1016/j.wneu.2024.01.167>
- Aderinto N, Abdulbasit MO, Olatunji D. Stem cell-based combinatorial therapies for spinal cord injury: a narrative review of current research and future directions. *Ann Med Surg (Lond).* 2023; 85(8):3943-3954. <https://doi.org/10.1097/MS9.0000000000001034>
- Khan SI, Ahmed N, Ahsan K, Abbasi M, Maugeri R, Chowdhury D, et al. An Insight into the Prospects and Drawbacks of Stem Cell Therapy for Spinal Cord Injuries: Ongoing Trials and Future Directions. *Brain Sci.* 2023; 13(12):1697. <https://doi.org/10.3390/brainsci13121697>
- Szymoniuk M, Litak J, Sakwa L, Dryla A, Zezuliński W, Czyżewski W, et al. Molecular Mechanisms and Clinical Application of Multipotent Stem Cells for Spinal Cord Injury. *Cells.* 2022; 12(1):120. <https://doi.org/10.3390/cells12010120>
- Shang Z, Wang M, Zhang B, Wang X, Wanyan P. Clinical translation of stem cell therapy for spinal cord injury still premature: results from a single-arm meta-analysis based on 62 clinical trials. *BMC Med.* 2022; 20(1):284. <https://doi.org/10.1186/s12916-022-02482-2>
- Woerly S, Plant GW, Harvey AR. Cultured rat neuronal and glial cells entrapped within hydrogel polymer matrices: a potential tool for neural tissue replacement. *Neurosci Lett.* 1996; 205(3):197-201. [https://doi.org/10.1016/0304-3940\(96\)12349-1](https://doi.org/10.1016/0304-3940(96)12349-1)

20. Woerly S, Doan VD, Evans-Martin F, Paramore CG, Peduzzi JD. Spinal cord reconstruction using NeuroGel implants and functional recovery after chronic injury. *J Neurosci Res*. 2001; 66(6):1187-97. <https://doi.org/10.1002/jnr.1255>
21. Woerly S, Doan VD, Sosa N, de Vellis J, Espinosa A. Reconstruction of the transected cat spinal cord following NeuroGel implantation: axonal tracing, immunohistochemical and ultrastructural studies. *Int J Dev Neurosci*. 2001; 19(1):63-83. [https://doi.org/10.1016/s0736-5748\(00\)00064-2](https://doi.org/10.1016/s0736-5748(00)00064-2)
22. Woerly S, Doan VD, Soszhanga N, de Vellis J, Espinosa-Jeffrey A. Prevention of gliotic scar formation by NeuroGel allows partial endogenous repair of transected cat spinal cord. *J Neurosci Res*. 2004; 75(2):262-272. <https://doi.org/10.1002/jnr.10774>
23. Pertici V, Amendola J, Laurin J, Gignes D, Madaschi L, Carelli S, et al. The use of poly(N-[2-hydroxypropyl]-methacrylamide) hydrogel to repair a T10 spinal cord hemisection in rat: a behavioural, electrophysiological and anatomical examination. *ASN Neuro*. 2013 May 30;5(2):149-66. <https://doi.org/10.1042/AN20120082>
24. Abdallah I, Medvediev V, Draguntsova N, Voitenko N, Tsymbaliuk V. Dependence of the restorative effect of Macroporous poly(N-[2-Hydroxypropyl]-methacrylamide hydrogel on the severity of experimental lacerative spinal cord injury. *USMJ*. 2021; 127(4):8-21. [https://doi.org/10.32345/USMYJ.4\(127\).2021.8-21](https://doi.org/10.32345/USMYJ.4(127).2021.8-21)
25. Rybachuk O, Savvitska N, Pinet É, Yaminsky Y, Medvediev V. Heterogeneous pHMA hydrogel promotes neuronal differentiation of bone marrow derived stromal cells in vitro and in vivo. *Biomed Mater*. 2023; 18(1). <https://doi.org/10.1088/1748-605X/acadc3>
26. Rybachuk O, Nesterenko Y, Pinet É, Medvediev V, Yaminsky Y, Tsymbaliuk V. Neuronal differentiation and inhibition of glial differentiation of murine neural stem cells by pHMA hydrogel for the repair of injured spinal cord. *Exp Neurol*. 2023; 368:114497. <https://doi.org/10.1016/j.expneurol.2023.114497>
27. Petriv T, Rybachuk O, Vorodi M, Zarovna H, Luzan B, Medvediev V, et al. Tissue Engineering Approach Using Heterogeneous Hydrogel Combined with Umbilical Cord Derived Multipotent Stem Cells for the Consequences Military Spinal Cord Injury Neurosurgical Treatment (Case Report). *Stem Cells Transl Med*. 2024; 13(1):S16. <https://doi.org/10.1093/stctm/szae062.016>
28. Hou S, Rabchevsky AG. Autonomic consequences of spinal cord injury. *Compr Physiol*. 2014 Oct;4(4):1419-53. <https://doi.org/10.1002/cphy.c130045>
29. D'Amico JM, Condliffe EG, Martins KJ, Bennett DJ, Gorassini MA. Recovery of neuronal and network excitability after spinal cord injury and implications for spasticity. *Front Integr Neurosci*. 2014; 8:36. <https://doi.org/10.3389/fnint.2014.00036>. Erratum in: *Front Integr Neurosci*. 2014; 8:49.
30. Vierck C. Mechanisms of Below-Level Pain Following Spinal Cord Injury (SCI). *J Pain*. 2020; 21(3-4):262-280. <https://doi.org/10.1016/j.jpain.2019.08.007>
31. Gong H, Zhang ZY, Duan ZX, Mao XA, Wu YY, Rao JS, et al. Mechanisms of Different Motor Neurons in the Occurrence of Spasticity After Spinal Cord Injury: A Narrative Review. *Int J Mol Sci*. 2025; 26(11):5162. <https://doi.org/10.3390/ijms26115162>
32. Kopach O, Medvediev V, Krotov V, Borisuyk A, Tsymbaliuk V, Voitenko N. Opposite, bidirectional shifts in excitation and inhibition in specific types of dorsal horn interneurons are associated with spasticity and pain post-SCI. *Sci Rep*. 2017; 7(1):5884. <https://doi.org/10.1038/s41598-017-06049-7>
33. Finnerup NB. Neuropathic pain and spasticity: intricate consequences of spinal cord injury. *Spinal Cord*. 2017; 55(12):1046-1050. <https://doi.org/10.1038/sc.2017.70>
34. Shiao R, Lee-Kubli CA. Neuropathic Pain After Spinal Cord Injury: Challenges and Research Perspectives. *Neurotherapeutics*. 2018; 15(3):635-653. <https://doi.org/10.1007/s13311-018-0633-4>
35. Malcangio M. Role of the immune system in neuropathic pain. *Scand J Pain*. 2019 Dec 18; 20(1):33-37. <https://doi.org/10.1515/sjpain-2019-0138>
36. Le Blon D, Hoornaert C, Detrez JR, Bevers S, Daans J, Goossens H, et al. Immune remodelling of stromal cell grafts in the central nervous system: therapeutic inflammation or (harmless) side-effect? *J Tissue Eng Regen Med*. 2017; 11(10):2846-2852. <https://doi.org/10.1002/term.2188>
37. Han X, Liao R, Li X, Zhang C, Huo S, Qin L, et al. Mesenchymal stem cells in treating human diseases: molecular mechanisms and clinical studies. *Signal Transduct Target Ther*. 2025; 10(1):262. <https://doi.org/10.1038/s41392-025-02313-9>
38. Medvediev VV, Abdallah IM, Draguntsova NG, Savosko SI, Vaslovych VV, Tsymbaliuk VI, et al. Model of spinal cord lateral hemi-excision at the lower thoracic level for the tasks of reconstructive and experimental neurosurgery. *Ukr Neurosurg J*. 2021; 27(3):33-5. <https://doi.org/10.25305/unj.234154>
39. Trawczynski M, Liu G, David BT, Fessler RG. Restoring Motor Neurons in Spinal Cord Injury With Induced Pluripotent Stem Cells. *Front Cell Neurosci*. 2019; 13:369. <https://doi.org/10.3389/fncel.2019.00369>
40. Wang Y, Lv HQ, Chao X, Xu WX, Liu Y, Ling GX, et al. Multimodal therapy strategies based on hydrogels for the repair of spinal cord injury. *Mil Med Res*. 2022; 9(1):16. <https://doi.org/10.1186/s40779-022-00376-1>
41. Bryson JB, Kourgiantaki A, Jiang D, Demosthenous A, Greensmith L. An optogenetic cell therapy to restore control of target muscles in an aggressive mouse model of amyotrophic lateral sclerosis. *Elife*. 2024; 12:RP88250. <https://doi.org/10.7554/eLife.88250>
42. Raineteau O, Schwab ME. Plasticity of motor systems after incomplete spinal cord injury. *Nat Rev Neurosci*. 2001; 2(4):263-73. <https://doi.org/10.1038/35067570>
43. Koffler J, Zhu W, Qu X, Platoshyn O, Dulin JN, Brock J, et al. Biomimetic 3D-printed scaffolds for spinal cord injury repair. *Nat Med*. 2019; 25(2):263-269. <https://doi.org/10.1038/s41591-018-0296-z>
44. Mattucci S, Speidel J, Liu J, Kwon BK, Tetzlaff W, Oxland TR. Basic biomechanics of spinal cord injury - How injuries happen in people and how animal models have informed our understanding. *Clin Biomech (Bristol)*. 2019; 64:58-68. <https://doi.org/10.1016/j.clinbiomech.2018.03.020>
45. Quadri SA, Farooqui M, Ikram A, Zafar A, Khan MA, Suriya SS, et al. Recent update on basic mechanisms of spinal cord injury. *Neurosurg Rev*. 2020; 43(2):425-441. <https://doi.org/10.1007/s10143-018-1008-3>
46. Lee SW, Werner B, Park H, DeAndrea J, Ayutyanont N, York H. Epidemiology of demographic, clinical characteristics and hospital course of patients with spinal cord injury associated with vertebral fracture in a large private health care system in the United States. *J Spinal Cord Med*. 2024; 47(6):933-943. <https://doi.org/10.1080/10790268.2023.2228582>
47. Guijarro-Belmar A, Varone A, Baltzer MR, Kataria S, Tanriver-Ayder E, Watzlawick R, et al. Effectiveness of biomaterial-based combination strategies for spinal cord repair - a systematic review and meta-analysis of preclinical literature. *Spinal Cord*. 2022; 60(12):1041-1049. <https://doi.org/10.1038/s41393-022-00811-z>
48. Khan FI, Ahmed Z. Experimental Treatments for Spinal Cord Injury: A Systematic Review and Meta-Analysis. *Cells*. 2022; 11(21):3409. <https://doi.org/10.3390/cells11213409>
49. Pajer K, Bellák T, Nográdi A. Stem Cell Secretome for Spinal Cord Repair: Is It More than Just a Random Baseline Set of Factors? *Cells*. 2021; 10(11):3214. <https://doi.org/10.3390/cells10113214>
50. Fan XL, Zhang Y, Li X, Fu QL. Mechanisms underlying the protective effects of mesenchymal stem cell-based therapy. *Cell Mol Life Sci*. 2020; 77(14):2771-2794. <https://doi.org/10.1007/s00018-020-03454-6>
51. Hernández R, Jiménez-Luna C, Perales-Adán J, Perazzoli G, Melguizo C, Prados J. Differentiation of Human Mesenchymal Stem Cells towards Neuronal Lineage: Clinical Trials in Nervous System Disorders. *Biomol Ther (Seoul)*. 2020; 28(1):34-44. <https://doi.org/10.4062/biomolther.2019.065>
52. Bueno C, Martínez-Morga M, García-Bernal D, Moraleta JM, Martínez S. Differentiation of human adult-derived stem cells towards a neural lineage involves a dedifferentiation event prior to differentiation to neural phenotypes. *Sci Rep*. 2021; 11(1):12034. <https://doi.org/10.1038/s41598-021-91566-9>
53. George S, Hamblin MR, Abrahamse H. Differentiation of Mesenchymal Stem Cells to Neuroglia: in the Context of Cell Signalling. *Stem Cell Rev Rep*. 2019; 15(6):814-826. <https://doi.org/10.1007/s12015-019-09917-z>
54. Dörnen J, Dittmar T. The Role of MSCs and Cell Fusion in Tissue Regeneration. *Int J Mol Sci*. 2021; 22(20):10980. <https://doi.org/10.3390/ijms222010980>
55. Lee EJ, Lee MJ, Ryu YJ, Nam SH, Kim R, Song S, et al. Neuroplasticity therapy using glia-like cells derived from human mesenchymal stem cells for the recovery of cerebral infarction sequelae. *Mol Ther*. 2025; 33(1):356-374. <https://doi.org/10.1016/j.yth.2024.11.022>
56. Li W, Liu X, Li J. Progress of bone marrow mesenchymal stem cell transplantation on neural plasticity in brain. *Front Cell Dev Biol*. 2025; 13:1589169. <https://doi.org/10.3389/fcell.2025.1589169>

57. Duan YY, Chai Y, Zhang NL, Zhao DM, Yang C. Microtubule Stabilization Promotes Microcirculation Reconstruction After Spinal Cord Injury. *J Mol Neurosci*. 2021; 71(3):583-595. <https://doi.org/10.1007/s12031-020-01679-5>
58. Wang R, Bai J. Pharmacological interventions targeting the microcirculation following traumatic spinal cord injury. *Neural Regen Res*. 2024; 19(1):35-42. <https://doi.org/10.4103/1673-5374.375304>
59. Blesch A, Tuszynski MH. Spinal cord injury: plasticity, regeneration and the challenge of translational drug development. *Trends Neurosci*. 2009; 32(1):41-7. <https://doi.org/10.1016/j.tins.2008.09.008>
60. Shang Z, Li D, Chen J, Wang R, Wang M, Zhang B, et al. What Is the Optimal Timing of Transplantation of Neural Stem Cells in Spinal Cord Injury? A Systematic Review and Network Meta-Analysis Based on Animal Studies. *Front Immunol*. 2022 a; 13:855309. <https://doi.org/10.3389/fimmu.2022.855309>
61. Shang Z, Wang R, Li D, Chen J, Zhang B, Wang M, et al. Spinal Cord Injury: A Systematic Review and Network Meta-Analysis of Therapeutic Strategies Based on 15 Types of Stem Cells in Animal Models. *Front Pharmacol*. 2022 b; 13:819861. <https://doi.org/10.3389/fphar.2022.819861>
62. Biktimirov A, Bryukhovetskiy I, Sharma A, Sharma HS. Spinal cord stimulation and intrathecal baclofen therapy for patients with severe spasticity after spinal cord injury. *Prog Brain Res*. 2020; 258:79-99. <https://doi.org/10.1016/bs.pbr.2020.09.007>
63. Tamburin S, Filippetti M, Mantovani E, Smania N, Picelli A. Spasticity following brain and spinal cord injury: assessment and treatment. *Curr Opin Neurol*. 2022; 35(6):728-740. <https://doi.org/10.1097/WCO.0000000000001114>
64. Massey S, Vanhoestenbergh A, Duffell L. Neurophysiological and clinical outcome measures of the impact of electrical stimulation on spasticity in spinal cord injury: Systematic review and meta-analysis. *Front Rehabil Sci*. 2022; 3:1058663. <https://doi.org/10.3389/fresc.2022.1058663>
65. Jung Y, Breitbart S, Malvea A, Bhatia A, Ibrahim GM, Gorodetsky C. Epidural Spinal Cord Stimulation for Spasticity: a Systematic Review of the Literature. *World Neurosurg*. 2024; 183:227-238.e5. <https://doi.org/10.1016/j.wneu.2023.12.158>
66. Migliorini F, Cocconi F, Schäfer L, Simeone F, Jeyaraman M, Maffulli N. Pharmacological management of secondary chronic spinal cord injury: a systematic review. *Br Med Bull*. 2024; 151(1):49-68. <https://doi.org/10.1093/bmb/ldae009>
67. Wieters F, Weiss Lucas C, Gruhn M, Büschges A, Fink GR, Aswendt M. Introduction to spasticity and related mouse models. *Exp Neurol*. 2021; 335:113491. <https://doi.org/10.1016/j.expneurol.2020.113491>
68. Fouad K, Popovich PG, Kopp MA, Schwab JM. The neuroanatomical-functional paradox in spinal cord injury. *Nat Rev Neurol*. 2021; 17(1):53-62. <https://doi.org/10.1038/s41582-020-00436-x>
69. Kumar R, Lim J, Mekary RA, Rattani A, Dewan MC, Sharif SY, et al. Traumatic Spinal Injury: Global Epidemiology and Worldwide Volume. *World Neurosurg*. 2018; 113:e345-e363. <https://doi.org/10.1016/j.wneu.2018.02.033>
70. Krotov V, Medvediev V, Abdallah I, Bozhenko A, Tatarchuk M, Ishchenko Y, et al. Phenotypes of Motor Deficit and Pain after Experimental Spinal Cord Injury. *Bioengineering*. 2022; 9(6):262. <https://doi.org/10.3390/bioengineering9060262>
71. Badner A, Siddiqui AM, Fehlings MG. Spinal cord injuries: how could cell therapy help? *Expert Opin Biol Ther*. 2017; 17: 529-541. <https://doi.org/10.1080/14712598.2017.1308481>
72. Liu S, Xie YY, Wang B. Role and prospects of regenerative biomaterials in the repair of spinal cord injury. *Neural Regen Res*. 2019; 14:1352-1363. <https://doi.org/10.4103/1673-5374.253512>
73. Zhang Q, Shi B, Ding J, Yan L, Thawani JP, Fu C, et al. Polymer scaffolds facilitate spinal cord injury repair. *Acta Biomater*. 2019; 88:57-77. <https://doi.org/10.1016/j.actbio.2019.01.056>
74. Dohle E, Swanson E, Jovanovic L, Yusuf S, Thompson L, Horsfall HL, et al. Toward the Clinical Translation of Implantable Brain-Computer Interfaces for Motor Impairment: Research Trends and Outcome Measures. *Adv Sci (Weinh)*. 2025; 12(32):e01912. <https://doi.org/10.1002/advs.202501912>
75. Khan S, Kallis L, Mee H, El Hadwe S, Barone D, Hutchinson P, et al. Invasive Brain-Computer Interface for Communication: A Scoping Review. *Brain Sci*. 2025; 15(4):336. <https://doi.org/10.3390/brainsci15040336>
76. Dijkers MP, Akers KG, Dieffenbach S, Galen SS. Systematic Reviews of Clinical Benefits of Exoskeleton Use for Gait and Mobility in Neurologic Disorders: A Tertiary Study. *Arch Phys Med Rehabil*. 2021; 102(2):300-313. <https://doi.org/10.1016/j.apmr.2019.01.025>
77. Boufidis D, Garg R, Angelopoulos E, Cullen DK, Vitale F. Bio-inspired electronics: Soft, biohybrid, and "living" neural interfaces. *Nat Commun*. 2025; 16(1):1861. <https://doi.org/10.1038/s41467-025-57016-0>



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Вплив імплантації РНРМА-гідрогелю з мезенхімальними стромальними клітинами людини різного походження на відновлення рухової функції кінцівок у щурів з травмою спинного мозку

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РЕЗЮМЕ

Травма спинного мозку — доволі часте ушкодження в умовах бойових дій, що, окрім істотного збільшення ризику смерті, у більшості випадків спричиняє пожиттєві порушення рухових та інших функцій. Відновне лікування цього виду травми є однією з найтяжчих проблем сучасної медицини, вирішення якої пов'язують із застосуванням біоінженерних імплантів у комбінації з стовбуровими клітинами, які б сприяли регенераційному росту аксонів у денервовану частину спинного мозку.

МЕТА — встановити вплив імплантації РНРМА-гідрогелю з мезенхімальними стромальними клітинами людини різного походження на відновлення рухової функції кінцівок у щурів після моделювання травми спинного мозку.

МАТЕРІАЛИ І МЕТОДИ. Травму спинного мозку моделювали на 70 самцях білих безпородних щурів віком 3–4 міс. шляхом лівобічного висічення фрагменту половини поперечника спинного мозку на грудно-поперековому рівні. Для імплантації в зону травми використовували РНРМА гідрогель (HG) самостійно або попередньо заселений мультипотентними мезенхімальними стромальними клітинами стінки пуповинної артерії (MSC-UA) чи дерми людини (MSC-Dr). Контрольну групу склали тварини із змодельованим ушкодженням без лікування. Рухову активність і спастичність у паретичній кінцівці оцінювали на 7-му, 14-ту добу та щомісяця протягом 4 міс. після травми за шкалою Basso-Beattie-Bresnahan (BBB) та Ashworth, відповідно.

РЕЗУЛЬТАТИ. Через 4 міс. після травми найбільші значення показника функції спостерігали у групі MSC-UA, найменші — у групі MSC-Dr та проміжні — у решті груп. Різниця виявилася достовірною між групами контролю і MSC-UA та між MSC-UA і MSC-Dr ($p < 0.05$). Істотне збільшення показника функції у групі контролю відмічали протягом 2-х місяців після травми, у групі HG — протягом одного місяця, у групах MSC-Dr та MSC-UA — протягом 4-х міс. після травми.

Через 4 міс. після моделювання найбільші значення показника спастичності спостерігали у групі MSC-Dr, найменші — у групах контролю і MSC-UA, причому достовірні відмінності встановлено лише між групами MSC-UA і MSC-Dr ($p < 0.02$). Крім того, у всіх групах виявили істотну негативну кореляцію між індивідуальними значеннями ПФ і ПС, найсильнішу у групах MSC-Dr і SCI.

ВИСНОВОК. Імплантація у зону дефекту спинного мозку щура РНРМА-гідрогелю, заселеного мезенхімальними стромальними клітинами стінки пуповинної артерії людини, сприяє покращенню відновлення рухової функції паретичної кінцівки на 4 міс спостереження, хоча і достовірно не впливає на спастичність. При застосуванні гідрогелю без клітин, або заселеного МСК дерми не встановлено достовірного позитивного впливу на локомоторну активність та спастичність паретичної кінцівки щура.

КЛЮЧОВІ СЛОВА: травма спинного мозку; РНРМА-гідрогель; мезенхімальні стромальні клітини; рухова функція кінцівки; спастичність