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Impact of Nanocellulose-Based Biocomposite Implantation on Rodent Behavior in the Elevated Plus Maze Test

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ABSTRACT

Introduction: For application in regenerative medication, the biomaterial used in implants should have both structural and biological compatibility along with long-term safety with respect to the central nervous system. Nanocellulose (NC) is a promising biomaterial when it comes to biocompatibility, mechanical strength, and modifiability. However, there is a limited data on its long-term behavioural effects. Assessment of animal behaviour is an important aspect of preclinical safety evaluation.

Methods: The six-month in vivo study was conducted using 20 male rats divided into an experimental group with intramedullary implantation of a nanocellulose-based biocomposite and a control group with an autologous blood clot. The elevated plus maze test was used to assess the behavioural activity, which includes anxiety-related behaviour, exploratory activity, emotional responses, and locomotor function.

Results: No statistically significant differences were observed between the experimental and control groups for any of the analysed behavioural parameters. The animals exhibited comparable levels of anxiety, emotional state, and locomotor activity, with no signs of increased stress or neurotoxic effects. The findings show the implantation of the nanocellulose-based biocomposite does not cause adverse long-term behavioural effects in rats, supporting its biocompatibility and behavioural safety.

Conclusion: These findings are consistent with current concepts of nanocellulose biocompatibility and support the potential for its further application in regenerative medicine and tissue engineering.

Keywords: nanocellulose, toxicity, biocomposite, bone, elevated plus maze.

Introduction

Assessment of the biocompatibility and safety of novel biomaterials is a key stage in preclinical research, especially when they are intended for use as implantable materials [1, 2]. Along with morphological, biochemical, and histological methods, the analysis of the behavioral responses in laboratory animals is important, as it allows the identification of possible long-term neurotoxic or stress-induced effects of experimental interventions [3].

Nanocellulose is regarded as a promising material for use in regenerative medicine and tissue engineering due to its biocompatibility, mechanical strength, porosity, and modifiability. It is used for tissue regeneration, the creation of dressings, targeted drug delivery and the production of implants [4, 5]. However, the implantation of permanent biomaterials may potentially influence the overall physiological state of the organism, including the central nervous system, which requires a comprehensive in vivo safety assessment [6].

Behavioral tests in rodents are a sensitive tool for detecting changes in emotional state, anxiety, and motor activity. One of the most widely used methods is the elevated plus maze test, based on the natural conflict between an animal's desire for exploratory activity and its avoidance of open spaces. Analysis of the results of this test allows us to assess the level of anxiety, locomotor activity, and stress response in animals [7].

Hypothesis: Implantation of nanocellulose-based biocomposite does not cause an increase in anxiety-like behavior in rats in the elevated plus maze (EPM) test compared to the control group.

Aim: to evaluate behavioral responses in rats following implantation of a nanocellulose-based biocomposite using the elevated plus maze test.

Methods

Surgical procedure

The production of nanocellulose and the fabrication of a biocomposite incorporating calcium phosphate were conducted in collaboration with the Department of Physical and Colloid Chemistry. The number of animals is determined by the recommended groups from GOST for assessing the biological effect of chronic toxicity. The surgical intervention was performed on 20 male rats. The animals were divided into two experimental groups, with 10 rats in each group.

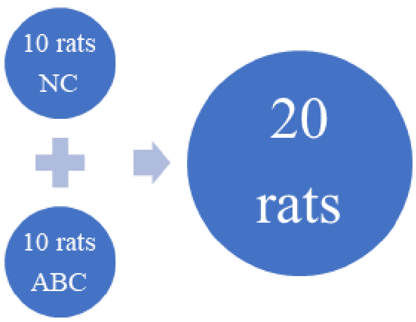


Figure 1 – Research design

Anesthesia was performed with Tiletamine (0.1 mg/kg). Implantation was performed in the middle third of the left femur. The first group included animals that underwent intramedullary implantation of nanocellulose into the femur (Figure 2). In the second group, the bone defect was filled with an autologous blood clot (ABC).

Figure 2 – Filling a defect in the femur with a nanocellulose-based biocomposite

All procedures related to animal housing, experimental manipulations, and analysis of the data obtained complied with the established standards of GOST ISO 10993-11—2021 and GOST ISO 10993-6—2021. The study was approved by a meeting of the local bioethics committee.

Elevated Plus Maze test

The study was conducted to assess the chronic toxicity of nanocellulose biocomposite. The observation period was 6 months. Prior to euthanasia, all animals underwent the elevated plus maze (EPM) test, which consists of two open arms and two dark closed arms arranged perpendicularly (Figure 3).

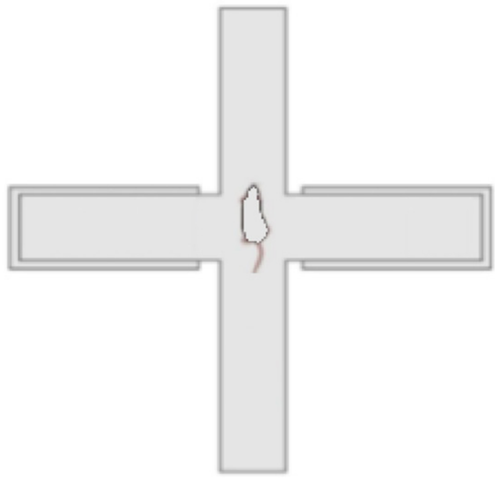


Figure 3 – Base of the elevated plus maze test: A – open arms, B – closed arms

Before each test, the maze was cleaned and wiped with alcohol. The camera was positioned to ensure full coverage of the maze. Before starting the video recording, animal identification was documented by displaying a card indicating the experimental group and the individual rat number.

Each animal was placed at the intersection of the open and closed arms, in the center of the maze, with its muzzle facing the open arm. The duration of video recording was five minutes. After completion of the test, the animal was removed and the maze was cleaned. A test protocol was completed, including the assessment of a range of parameters related to locomotor activity, anxiety and vegetative responses.

In the present study, the elevated plus maze test was used to assess the behavioral responses of rats six months after implantation of the nanocellulose-based biocomposite. The choice of a long-term observation period was determined by the need to identify potential chronic or delayed effects that may not be evident during the early postoperative period.

Statistical analysis

Statistical analysis of the study results was performed using STATISTICA 6.0 software (StatSoft, USA). Quantitative data were analyzed using standard methods of variation statistics. The results are presented as median and interquartile range. Differences between groups were considered statistically significant at $p < 0,05$. For pairwise comparisons, the nonparametric Mann–Whitney U test was also applied.

Results

There were no seizures during dynamic observation. The parameters assessed in the elevated plus maze (EPM) test are presented in Table 1.

Comparative analysis of rat behavior in the elevated plus maze 6 months after the intervention revealed no pronounced differences between the groups across all studied parameters.

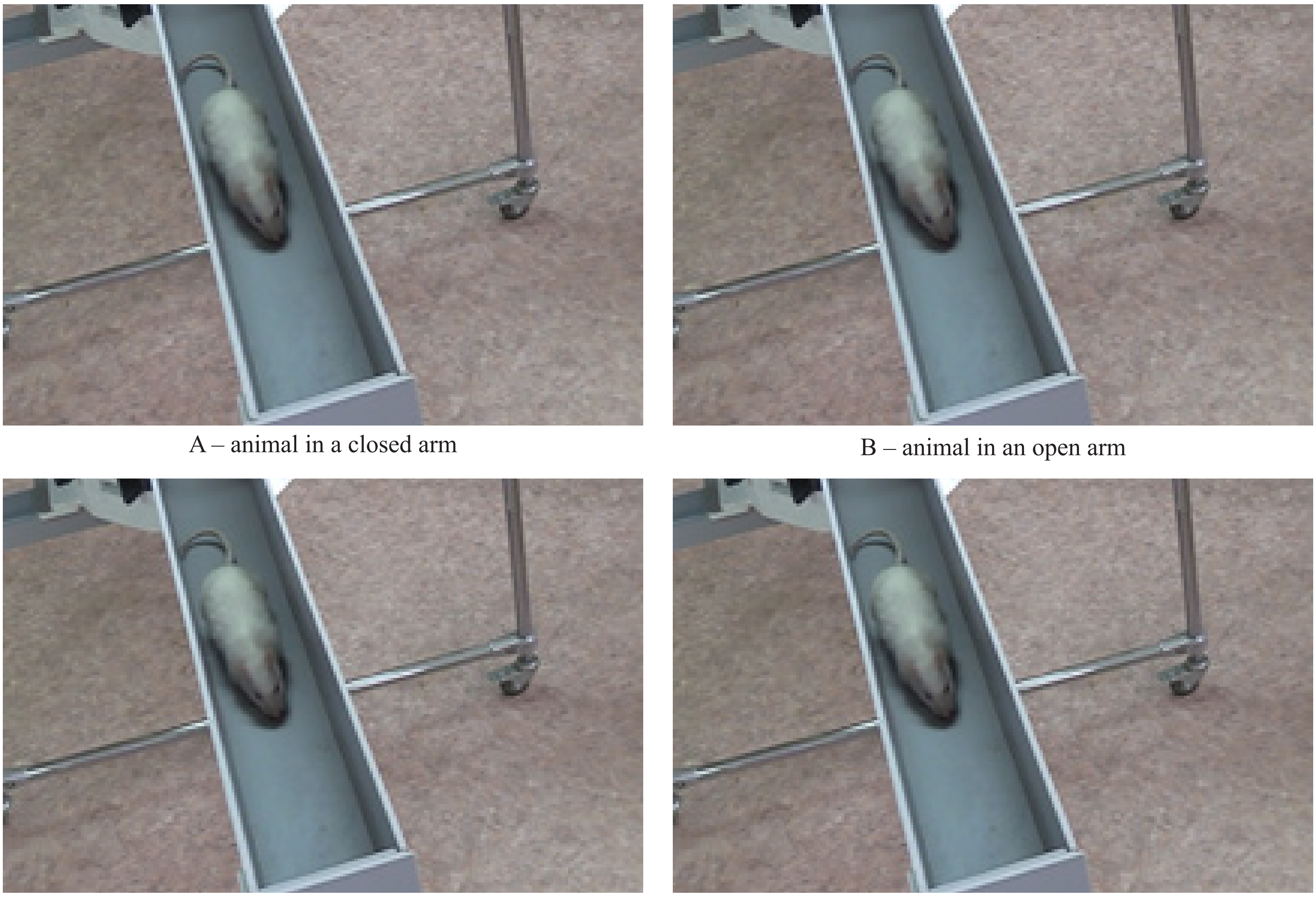
The latency to leave the central platform was identical in both groups, indicating no differences in baseline locomotor activity or orienting responses. The total time spent on the central platform, as well as in the closed and open arms of the maze, did not differ significantly, suggesting a comparable level of anxiety in animals from both groups (Figure 4A, B).

Indicators of exploratory activity, including the number of entries into closed arms and the centre, remained within physiological limits and did not differ between groups. A similar pattern was observed for the number of entries into open arms and the number of head dippings, indicating no effect of the intervention on anxiety-related or risk-associated behaviors (Figure 4C).

Markers of emotional tension (grooming behavior, number of fecal boli, and rearings) also showed no significant intergroup differences (Figure 4D). The absence of increased defecation and grooming in open arms indicates a lack of pronounced stress exposure. The number of squares crossed in both open and closed arms was comparable between groups, reflecting preserved locomotor activity.

Table 1 Behavioral responses in the elevated plus maze test

Parameter	Group 1 (NC) Me [Q1; Q3]	Group 2 (ABC) Me [Q1; Q3]	Mann–Whitney test
Latency to leave the central platform, s	0 [0; 0]	0 [0; 0]	$p > 0.05$
Total time spent on the central platform, s	0,5 [0; 1]	1 [0; 1]	$p > 0.05$
Total time spent in closed arms, s	280 [269; 300]	294 [283;299]	$p > 0.05$
Total time spent in open arms, s	13 [0; 20]	3 [0; 8]	$p > 0.05$
Number of entries into closed arms, times	4 [1; 4]	1 [1; 3]	$p > 0.05$
Number of entries into center, times	5 [0; 6]	1,5 [0; 2]	$p > 0.05$
Number of entries into open arms, times	1 [0; 2]	1 [0; 1]	$p > 0.05$
Number of head dippings in open arms, times	3 [0; 3]	0,5 [0; 2]	$p > 0.05$
Grooming in open arms, times	-	-	-
Grooming in closed arms, times	3 [3; 3]	3 [2; 5]	$p > 0.05$
Number of rearings in open arms, times	-	-	-
Number of rearings in closed arms, times	9 [7; 10]	5 [3; 9]	$p > 0.05$
Number of fecal boli (defecations), times	0 [0; 1]	0 [0; 0]	$p > 0.05$
Number of squares crossed in open arms, times	6 [0; 6]	1,5 [0; 4]	$p > 0.05$
Number of squares crossed in closed arms, times	18 [3; 29]	9 [5; 16]	$p > 0.05$



A – animal in a closed arm

B – animal in an open arm

C – head dipping in an open arm

D – rearing in a closed arm

Figure 4 – Selected behavioral responses in the elevated plus maze test

The obtained data indicate that the investigated intervention does not exert a significant effect on anxiety levels, emotional state, or motor activity of rats at long-term follow-up. The absence of statistically significant differences ($p > 0.05$) across key test parameters confirms the behavioral safety and lack of neurotoxic effects of the nanocellulose-based biocomposite.

Discussion

The elevated plus maze test is a validated method for assessing anxiety, emotional state, and locomotor activity in laboratory animals and is widely used to detect potential neurotoxic and behavioral effects of experimental interventions [7, 8, 9]. In the present study, this test was used to evaluate the long-term behavioral consequences of implantation of a nanocellulose-based biocomposite.

The results demonstrate the absence of a pronounced effect of the tested material on rat behavioral responses 6 months after implantation. Key anxiety-related parameters—time spent in open and closed arms and the number of entries into open arms—did not show statistically significant differences between the experimental and control groups. This suggests the absence of both anxiogenic and anxiolytic effects of the biocomposite implantation [10, 11, 12].

The lack of differences in latency to leave the central platform and total time spent in the maze center indicates that the intervention did not affect baseline locomotor activity or exploratory behavior. Comparable values for the number of squares crossed in open and closed arms further confirm the preservation of overall locomotor function and the absence of motor impairments that could confound anxiety assessment. These findings are consistent with previously published data [10, 12].

Markers of emotional tension, including grooming frequency, number of defecations, rearings, and head dippings, also did not differ significantly between groups. These parameters are considered additional indicators of stress response, and their stability suggests the absence of chronic stress or discomfort at long-term observation points. This is particularly important for evaluating the biocompatibility of implantable materials, as emphasized by multiple authors [10, 12, 13, 14].

It should be noted that quantitative fluctuations in individual parameters were variable and accompanied by relatively high standard deviations, which is characteristic of behavioral testing. However, the absence of statistically significant differences allows these variations to be interpreted as individual variability rather than effects of the experimental intervention, in agreement with the literature [15].

Conclusion

Thus, the results of behavioral testing in the elevated plus maze confirm the absence of adverse effects of the nanocellulose-based biocomposite on the central nervous system, anxiety level, and locomotor activity of rats at long-term follow-up after implantation. These findings are consistent with current concepts of nanocellulose biocompatibility and support the potential for its further application in regenerative medicine and tissue engineering.

Limitations

Therefore, the obtained results are preliminary and should be viewed with caution. This study has several limitations that should be considered when interpreting the findings. First, the sample size ($n = 10$ per group) was consistent with commonly used designs in experimental behavioral neuroscience; however, it may have provided limited statistical power, particularly for detecting small effect sizes. The use of the nonparametric Mann–Whitney U test, although appropriate given the distribution of the data, may further reduce sensitivity compared to parametric approaches.

Accordingly, the possibility of a type II error (β-error) cannot be excluded. The absence of statistically significant differences ($p > 0.05$) does not definitively rule out the presence of subtle between-group effects that may not have been detectable within the current sample size. Second, behavioral parameters in the Elevated Plus Maze test are characterized by inherent inter-individual variability, which may increase dispersion and reduce the ability to identify modest group differences. Third, behavioral assessment was conducted at a single post-implantation time point. Potential transient or delayed neurobehavioral effects could therefore remain undetected.

Future studies with larger sample sizes, predefined effect size assumptions, and longitudinal behavioral assessment are warranted to further validate the present findings. Further histological examination of the organs for toxicity is planned.

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