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Local cytokine changes following fibrin-encapsulated mesenchymal stem cell-derived extracellular vesicle therapy in rat spinal cord injury

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Abstract

Spinal cord injury (SCI) induces a prolonged and complex inflammatory response that contributes to secondary damage and influences functional recovery. Targeting this inflammatory milieu represents a promising therapeutic strategy. In this study, we investigated the effects of extracellular vesicles (EVs) derived from mesenchymal stem cells (MSCs) and encapsulated in a fibrin matrix (FM) on cytokine regulation in a rat model of SCI 60 days post-injury. MSCs were isolated from rat adipose tissue, and EVs were obtained using cytochalasin B-induced vesiculation. The EVs were encapsulated in FM and applied locally to the injury site at doses of 5 and 10 μg. The SCI rat models were divided into four groups: untreated, treated with FM alone, treated with 5 μg of EVs in FM (FM+EVs5), and treated with a 10 μg dose (FM+EVs10). A multiplex assay was employed to quantify the levels of 23 cytokines in spinal cord tissue homogenates. The application of FM alone altered cytokine levels, notably increasing granulocyte colony-stimulating factor (G-CSF) levels by 2.8-fold, which may be attributed to the hemostatic and bioactive properties of fibrin. Treatment with MSC-derived EVs resulted in a dose-dependent modulation of inflammatory responses. In the FM+EVs10 group, pro-inflammatory cytokines interleukin (IL)-1β and IL-5, as well as the anti-inflammatory cytokine IL-10, were significantly reduced compared to both the untreated and FM-alone groups, with IL-10 levels decreasing 2.4-fold. A similar trend was observed for IL-17A, which was 1.6-fold lower in the FM+EVs10 group compared to the FM-alone group. These findings suggest that fibrin-encapsulated MSC-derived EVs can modulate inflammation in chronic SCI and warrant further investigation as a therapeutic approach for neuroprotection and tissue repair.

Keywords: Spinal cord injury; Mesenchymal stem cells; Extracellular vesicles; Fibrin matrix; Cytokine modulation; Rats

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1. Introduction

Spinal cord injury (SCI) is one of the most serious public health concerns, as it often results in permanent disability. Primary mechanical damage to nerve tissue triggers a cascade of secondary pathological reactions, with neuroinflammation playing a key role. A crucial component of this process is the excessive production of pro-inflammatory cytokines, which exacerbates neural tissue damage. Analyses of cerebrospinal fluid and blood samples from patients with SCI have revealed pronounced alterations in the cytokine profile, which are important for predicting clinical outcomes and evaluating therapeutic efficacy.[1,2] Therefore, approaches to post-traumatic spinal cord repair should incorporate anti-inflammatory effects.

The use of mesenchymal stem cells (MSCs) and/or their paracrine mediators encapsulated in extracellular vesicles (MSC-EVs) is considered a promising therapeutic strategy. Studies using a rat model of SCI have shown that systemic administration of MSC-EVs reduces levels of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β, while increasing the production of anti-inflammatory molecules like IL-10. In this context, EVs regulate inflammatory factors through multiple mechanisms, such as the delivery of miRNAs and proteins.[3] In addition, beyond influencing apoptosis and neuroinflammation, systemic administration of MSC-EVs has also been shown to modulate angiogenesis, thereby promoting tissue healing by accelerating the elimination of inflammatory mediators and attracting immune cells to the injury site.[4]

A study by Romanelli *et al.* (2019) examined the long-term effects of intravenously administered EVs in a rat model of spinal contusion. The study found that during the chronic phase of SCI, both the experimental and control groups continued to lose neural tissue. However, by the end of the experiment, significantly more tissue was preserved in the EV-treated group compared to the control group.[5] In a follow-up study, intraspinal administration of EVs led to a more pronounced suppression of inflammatory responses, a reduction in pro-inflammatory cytokine release (TNF-α and IL-6), and an increase in anti-inflammatory cytokine production (IL-10).[6] In addition, this approach reduced scar tissue formation and improved motor function compared to systemic EV administration. This difference may be due to localized administration providing a more rapid and targeted effect on inflammatory processes at the injury site.[6] Despite being more invasive than intravenous injection, local delivery of cells or EVs near the spinal cord lesion has generally been shown to be safe when performed slowly and carefully in patients with SCI.[7]

In view of these findings, the local application of MSC-EVs may be an effective anti-inflammatory approach for SCI, offering targeted effect at the site of inflammation and reducing systemic side effects. However, their use requires the development of an optimal delivery system that ensures prolonged and controlled release of EVs at the injury site. One promising approach is the use of a fibrin matrix (FM), which is widely employed in clinical practice due to its biocompatibility and natural biodegradability. FM serves as a biocompatible and biodegradable scaffold that enables sustained release of encapsulated MSCs and their exosomes.[8-11] In addition, its intrinsic bioactivity may synergistically contribute to inflammation modulation, thereby improving the therapeutic efficacy of MSC-EVs.

Our previous study demonstrated that the application of MSC-EVs promoted the preservation of mature oligodendrocytes and improved functional outcomes in rats with SCI.[12] However, no studies have thoroughly evaluated the effects of combining MSC-EVs with FM on both pro-inflammatory and anti-inflammatory cytokine levels in SCI. Chronic inflammation persisting beyond the acute injury phase contributes to ongoing tissue damage and impedes functional recovery.[13-15] Targeting inflammatory processes at the chronic stage is therefore critical for promoting regenerative mechanisms and improving long-term outcomes. Understanding how therapies modulate inflammation during this phase is essential for developing effective treatments for SCI. The present study aimed to investigate the impact of co-administering MSC-EVs with FM on cytokine levels in the rat spinal cord during the chronic phase of SCI.

2. Materials and methods

All procedures were designed to minimize animal use and reduce the severity of interventions. The experimental design is summarized in Figure 1.

2.1. Isolation and cultivation of MSCs

MSCs were obtained from the adipose tissue of female Wistar rats weighing 250–300 g (Pushchino Laboratory, Russia). The rats were anesthetized using isoflurane (1.3%; Laboratories Karizoo, Spain) and Zoletil (20 mg/kg; Virbac, France) before undergoing surgery. Adipose tissue was carefully collected in a sterile environment and placed into a container with 0.9% sodium chloride (NaCl) solution (PanEco, Russia). The tissue was homogenized, centrifuged in 0.9% NaCl, and finely minced for 10 min at 1,500 rpm. Afterward, it was treated with a 0.5% collagenase solution derived from crab pancreas (Biolot, Russia) at 37°C for 1 h with constant shaking at 180 rpm. Following this, the homogenate was centrifuged at 1,400 rpm for 5 min to remove the enzyme solution. The remaining cells were washed in Dulbecco's phosphate-buffered saline (DPBS; PanEco, Russia), centrifuged again to remove residual

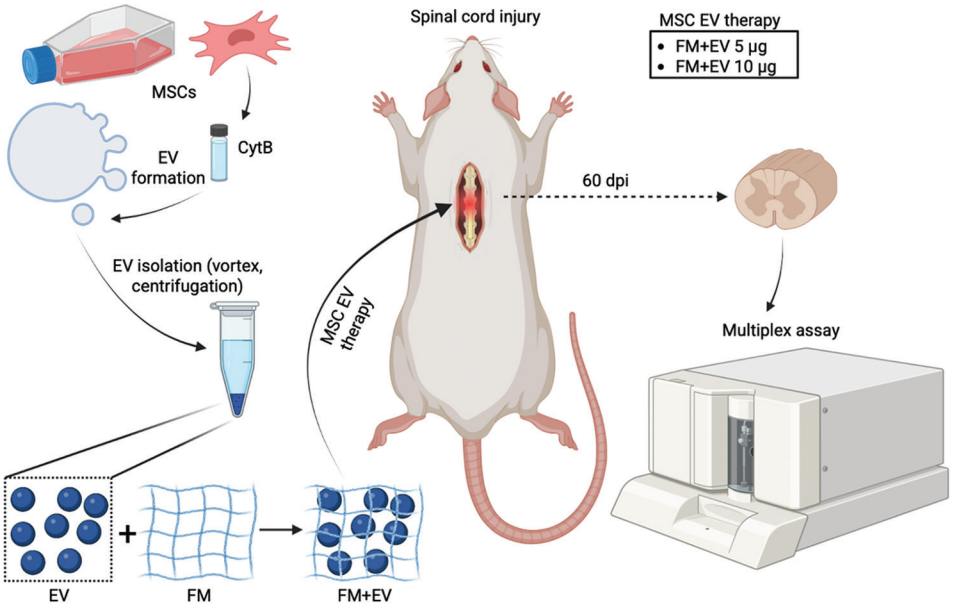

Figure 1. Experimental design of the study. Mesenchymal stem cells (MSCs) were isolated from rat adipose tissue and cultured. Extracellular vesicles (EVs) were isolated from MSCs using cytochalasin B (CytB). EVs, at doses of 5 µg and 10 µg, were then encapsulated in a fibrin matrix (FM) and applied to the site of SCI in rats. 60 days post-injury (dpi), the spinal cord was harvested, and multiplex analysis was performed to assess cytokine levels. Image created with Biorender.com.

enzymes, and prepared for culture. The cells were then grown in Dulbecco's modified Eagle medium (DMEM), enriched with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 µg/mL streptomycin, and 100 U/mL penicillin (all from PanEco, Russia). The culture medium was replaced every 3 days. Cells in the third passage were used for EV collection.

2.2. Isolation of EVs

Cells were grown until they reached 90–95% confluency. The growth medium was then aspirated, and the cells were rinsed with DPBS before detachment using 0.25% trypsin solution (PanEco, Russia). To neutralize the trypsin, DMEM containing 10% FBS was added. Following this, the cells were centrifuged at 1,400 rpm for 5 min. To remove any residual serum, the cells were washed with 0.9% NaCl. Next, the cells were incubated for 30 min in serum-free DMEM supplemented with 10 µg/mL cytochalasin B (Sigma-Aldrich, USA) at 37°C and 5% CO_2. After incubation, the cell suspension was vigorously mixed on a vortex for 60 s and then subjected to centrifugation at 500 rpm for 10 min. The supernatant was collected and further centrifuged at 700 rpm for 10 min, followed by a final centrifugation at 12,000 rpm for 15 min. The resulting pellet, containing EVs, was resuspended in 0.9% NaCl.

2.3. SCI and MSC-EVs therapy

The study was carried out on adult female Wistar rats (n = 23) weighing 250–300 g. Female rats were selected due to the relative ease of postoperative care, specifically the facilitated mechanical urination. The animals were housed in a 12-h light/dark cycle with food and water available *ad libitum*. Anesthesia was administered using isoflurane (1.3%) and zoletil (20 mg/kg) for all surgical procedures. Following a laminectomy, a moderate spinal cord contusion injury was induced at the Th8 level with an impact speed of 2.5 m/s using the Impact One Stereotaxic Impactor (Leica, Germany).

The preparation of the FM was performed according to the manufacturer's instructions. The commercial kit (Tissucol Kit, Baxter, USA) contains vials used to obtain a two-component glue. In brief, we obtained the first component by mixing fibrinogen with aprotinin, and the second component is obtained by mixing thrombin with calcium chloride. Equal quantities of each component were used. Specifically, 9 µL of fibrinogen in aprotinin was mixed with the EV suspension at 37°C. Immediately before application to the injury site, 9 µL of thrombin solution with calcium chloride was added to the fibrinogen-EV mixture, and the resulting solution was applied to the injury site using a mechanical pipette. EV doses were determined based on total protein concentration, quantified using the BCA Protein Assay Kit (Thermo Fisher Scientific, USA).

The animals were divided into four groups. In the first control group, animals received no therapy (SCI group: n = 6), while in the second control group, FM without

MSC-EVs was applied immediately after injury (SCI FM group: $n = 6$). In the two experimental groups, 5 and 10 μg of MSC-EVs encapsulated in 18 μl of FM (Tissucol®; Baxter, USA) were applied immediately after injury (SCI FM+EVs5 group: $n = 6$; SCI FM+EVs10 group: $n = 5$). After surgery, all rats received daily intramuscular gentamicin (25 mg/kg; Microgen, Russia) for 7 days. Bladders of the injured rats were manually emptied twice daily until spontaneous urination was restored.

2.4. Multiplex assay

To assess the cytokine profiles at 60-day post-injury (dpi), a section of the spinal cord, including the injury epicenter, was dissected at the Th8 level. The tissue was homogenized using an electric homogenizer in 300 μL of complete extraction buffer. Following centrifugation at 13,000 rpm for 20 min at 4°C, the soluble protein extract was collected and stored at −80°C until cytokine analysis. The cytokine profile was determined using multiplex analysis with xMAP Luminex technology, specifically the Bio-Plex Pro Rat Cytokine 23-Plex Immunoassay (12005641, Bio-Rad, USA). This assay allows for the quantification of 23 cytokines and chemokines, including granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage CSF (GM-CSF), growth-related oncogene/keratinocyte-derived chemokine (GRO/KC), interferon (IFN)-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12 (p70), IL-13, IL-17A, IL-18, macrophage CSF (M-CSF), monocyte chemoattractant protein-1, macrophage inflammatory protein (MIP)-1α, MIP-3α, RANTES, TNF-α, and vascular endothelial growth factor.

2.5. Statistical analysis

Data were analyzed using the Origin Pro software (version 2020 [9.7], OriginLab Corp., USA). Data are presented as mean values with standard deviation (SD) or standard error (SE). A normality test was conducted for all study groups. One-way analysis of variance (ANOVA) followed by Tukey's test was performed for multiple group comparisons. All analyses were performed in a blinded manner relative to the study groups. A value of $p<0.05$ was considered statistically significant.

3. Results

Cytokine levels in spinal cord homogenates from the four groups (SCI, SCI FM, SCI FM+EVs5, and SCI FM+EVs10) at 60 dpi revealed notable differences in the expression patterns. The data obtained through multiplex analysis (Table A1) were visualized as a heat map (Figure 2).

The expression levels of several cytokines were significantly elevated in the second control group (SCI FM) compared to the first control group (SCI). Notably, G-CSF showed the highest increase, with a 2.8-fold elevation ($p<0.05$) (Figure 3A). Other cytokines also exhibited significant increases, including GM-CSF (1.6-fold), IFN-γ (2.3-fold), IL-18 (1.2-fold), IL-2 (2-fold), IL-6 and IL-7 (both 2.4-fold), M-CSF (1.7-fold), and MIP-3α (2.3-fold).

For IL-1β, IL-5, and IL-10, the SCI FM group showed significantly higher mean concentrations compared with both the SCI group and the SCI FM+EVs5 group ($p<0.05$) (Figure 3B-D). The application of 10 μg EVs (SCI FM+EVs10) to the lesion area significantly reduced

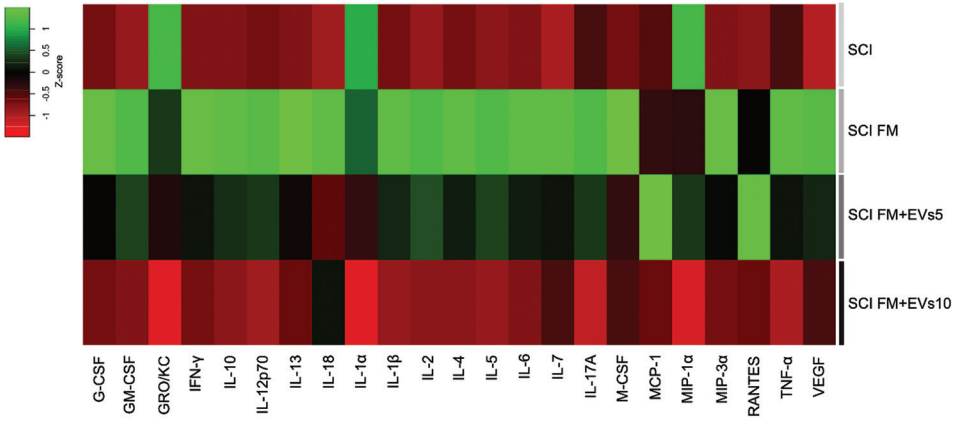

Figure 2. Multiplex analysis of spinal cord homogenates (Th8). The heat map shows upregulated (green) or downregulated (red) cytokines across four experimental groups: SCI (untreated), SCI FM (treated with FM only), SCI FM+EVs5 (treated with 5 μg EVs in FM), and SCI FM+EVs10 (treated with 10 μg EVs in FM).

Abbreviations: CSF: colony-stimulating factor; G-CSF: Granulocyte CSF; GM-CSF: Granulocyte–macrophage CSF; GRO/KC: Growth-related oncogene/keratinocyte-derived chemokine; EVs: Extracellular vesicles; FM: Fibrin matrix; IFN: Interferon; IL: Interleukin; M-CSF: macrophage CSF; MCP: Monocyte chemoattractant protein; MIP: Macrophage inflammatory protein; SCI: Spinal cord injury; TNF: Tumor necrosis factor; VEGF: Vascular endothelial growth factor.

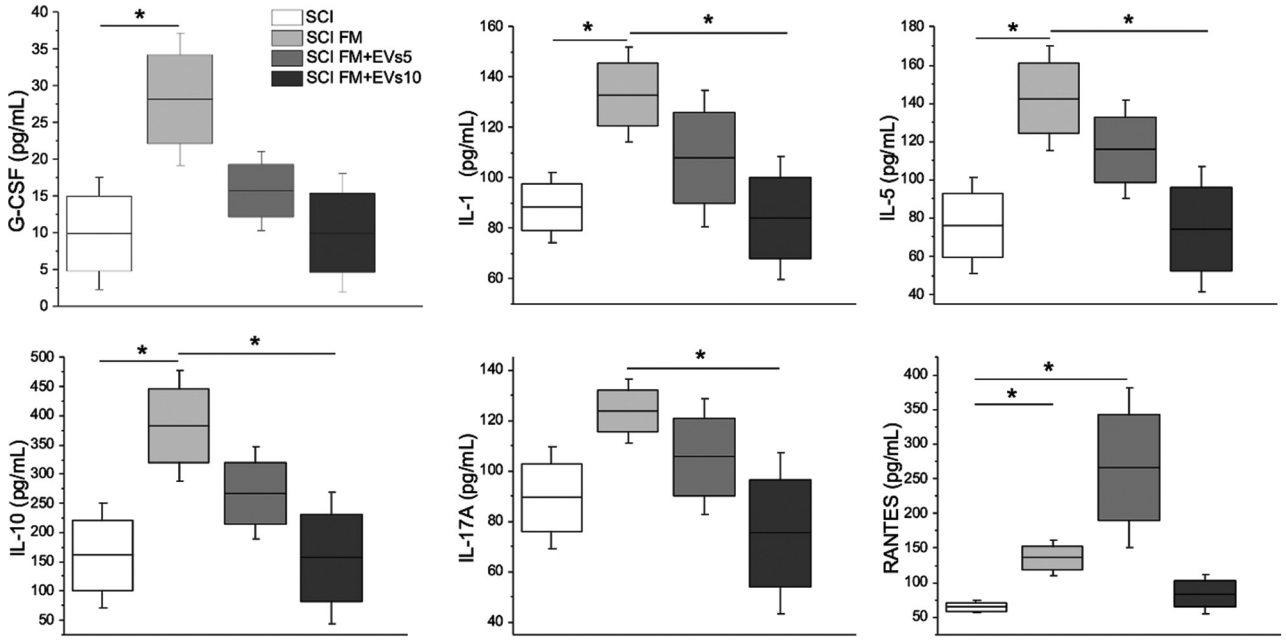

Figure 3. Comparison of cytokine levels. Boxplots display the concentrations of G-CSF, IL-10, IL-1β, IL-5, IL-17A, and RANTES across four experimental groups: SCI (untreated), SCI FM (treated with FM only), SCI FM+EVs5 (treated with 5 μg EVs in FM), and SCI FM+EVs10 (treated with 10 μg EVs in FM). *p<0.05, ANOVA with Tukey's *post hoc* test.
Abbreviations: G-CSF: Granulocyte colony-stimulating factor; EVs: Extracellular vesicles; FM: Fibrin matrix; IL: Interleukin; SCI: Spinal cord injury.

the concentrations of pro-inflammatory IL-1β (1.5-fold) and IL-5 (1.9-fold), as well as the anti-inflammatory IL-10 (2.4-fold) relative to SCI FM.

A similar trend was observed for IL-17A levels, where a 1.6-fold decrease (p<0.05) was found in the SCI FM+EVs10 group compared to the SCI FM control group (Figure 3E). Notably, RANTES expression (Figure 3F) was significantly higher in the SCI FM+EVs5 group than in the SCI group (4-fold, p<0.05). In addition, RANTES levels were 2-fold higher in SCI FM compared with the SCI group (p<0.05).

4. Discussion

In our previous study,[12] we thoroughly characterized MSC-EVs using transmission electron microscopy, which confirmed their uniform morphology and size distribution, and flow cytometry, which demonstrated that MSC-EVs share surface markers with their parental MSCs, including Sca-1, CD49e, and CD44. Functional assessments, including Basso, Beattie, and Bresnahan (BBB) locomotor testing, revealed a dose-dependent improvement in motor recovery, with significantly higher BBB scores in EV-treated groups compared to untreated SCI controls. Electrophysiological analysis showed that M-wave amplitudes were significantly higher in the same groups, while motor evoked potentials (MEP) and somatosensory evoked potentials (SSEP) indicated modulation of both central and peripheral conduction pathways. MEP registration frequency progressively decreased over time in all SCI groups, whereas SSEP amplitudes showed preserved peripheral responses but impaired cortical signals. Histological and morphometric analyses revealed greater tissue preservation and oligodendrocyte survival in EV-treated groups, with a more pronounced neuroprotective effect at higher doses. These findings provide a strong foundation for the present investigation into the inflammatory responses following MSC-EV application in the same SCI model.

We have previously shown that MSCs embedded in an FM can retain their viability at the SCI site for up to 60 days and migrate rostrally and caudally for more than 5 mm.[16] In this study, we conducted a multiplex analysis of 23 cytokines in rat spinal cord homogenates at 60 dpi—representing the chronic phase of SCI—after the treatment with FM combined with MSC-EVs at two dosages (5 μg and 10 μg). The results were compared to SCI without treatment and SCI treated with FM alone. Our analysis revealed an upregulation of several cytokines following MSC-EV therapy.

FM treatment alone was associated with a sustained increase of multiple chemokines (G-CSF, M-CSF, GM-CSF) and predominantly pro-inflammatory effects (IL-1β, IL-2, IL-5, IL-6, IL-7, IL-17A, IL-18, IFN-γ, and MIP-3α),

alongside a more moderate rise in the anti-inflammatory cytokine IL-10. Cytokines such as GM-CSF, IFN-γ, and IL-6 were particularly elevated, possibly reflecting the intrinsic hemostatic properties of FM. Notably, IL-6—increased 2.4-fold in the FM group—was partially normalized in both EV-treated groups, particularly in FM+EVs10, indicating that EVs counteracted FM-induced upregulation of inflammatory cytokines. This supports the role of MSC-EVs in fine-tuning the local cytokine milieu, underscoring their capacity to modulate complex immune responses in the chronic phase of SCI. Previous studies indicate that fibrin glues, such as Tisseel® (also referred to as Tissucol® in our study), despite their hemostatic properties, can elicit local inflammatory responses.[17,18] In one study, fibrin glue application in a rat SCI model did not reduce inflammation, an effect likely linked to activation of coagulation-dependent inflammatory cascades.[17] This may explain the elevated levels of pro-inflammatory cytokines observed in our experiment. In addition, a study comparing Tisseel® with other adhesives, such as BioGlue® and Adherus®, demonstrated that Tisseel® caused relatively less pronounced inflammatory and degenerative responses in rat SCI.[18] However, even in these cases, localized inflammatory reactions were still observed, aligning with our findings. It is noteworthy that these studies examined the effects of adhesives on inflammation up to 28 days post-application, whereas our study extended to 60 dpi, suggesting that FM-induced inflammatory responses can persist into the chronic stage of SC.

The study of FM biodegradation in the body is also noteworthy. As a biocompatible material, Tisseel® is slowly resorbed in tissues, which may lead to prolonged interactions with surrounding cells and potentially sustain an inflammatory response. Research on various commercial fibrin matrices, including Tisseel®, has shown that the presence of protease inhibitors like aprotinin slows its biodegradation, suggesting that its extended presence in tissues may have prolonged effects on cellular responses.[19] In an *in vitro* study, Tisseel® was also found to increase metalloproteinases (MMP-1, MMP-2) levels in mesothelial cells and fibroblasts, which may alter cytokine profiles and prolong inflammation during matrix degradation.[20] Furthermore, studies have identified key cellular targets and molecular mechanisms by which fibrinogen and thrombin, essential components of Tisseel®, can hinder neurotrauma recovery. For instance, fibrinogen binding to αVβ3 inhibits neurite outgrowth in the central nervous system (CNS), as demonstrated in a mouse model of encephalomyelitis,[21] while its interaction with CD11b/CD18 activates microglia in a mouse model of traumatic brain injury.[22] It is plausible that MSC-EVs may attenuate FM-associated inhibitory signals by modulating integrin signaling pathways, such as reducing fibrinogen interaction with αVβ3 receptors or regulating CD11b/CD18-mediated microglial activation. EV cargo, including regulatory miRNAs or surface molecules, could contribute to these modulatory effects, potentially influencing natural tissue repair processes following traumatic injuries. It is also important to note that differences in viral inactivation and processing methods between Tisseel® and Vistaseal® have been suggested to theoretically influence the inflammatory potential of Tisseel®. However, the clinical significance of these differences has not yet been fully established.[23]

Our study is among the first to explore the effects of FM combined with MSC-EVs on the cytokine profile during the chronic phase of SCI (60 dpi). While previous research has examined the role of MSC-EVs encapsulated in FM in promoting oligodendrogenesis and functional recovery,[24] as well as neurogenesis[25] in rodent models of chronic SCI, the impact of this combination on neuroinflammatory processes within the injured spinal cord has not been addressed. We found that MSC-EVs can mitigate cytokine shifts at the injury site induced by local FM exposure, exhibiting a clear dose-dependent effect. Specifically, increasing the MSC-EV dosage significantly reduced the expression levels of IL-1β, IL-5, IL-17A, and IL-10 compared to the FM-only group. However, the notably elevated RANTES (CCL5) levels observed in the SCI FM+EVs5 group, as opposed to the FM+EVs10 group, warrant further investigation to clarify its role in anti-inflammatory or regenerative processes. This increase may reflect an insufficient dose of EVs to fully suppress specific pro-inflammatory pathways or could represent a transitional state in immune modulation. Given the pleiotropic functions of RANTES in both promoting and resolving inflammation depending on context, its dose-dependent dynamics in response to EV therapy are of particular interest for future studies.

A key point of comparison with our previous results is the dose-dependent immunomodulatory effect of MSC-EVs. The application of 10 μg MSC-EVs (SCI FM+EVs10) led to a significant reduction in both pro-inflammatory cytokines (IL-1β, IL-5, IL-17A) and the anti-inflammatory cytokine IL-10. This is consistent with our prior study,[12] where higher EV doses resulted in greater tissue preservation and enhanced electrophysiological recovery (M-wave, MEP, and SSEP measurements), indicating that MSC-EVs do not merely suppress inflammation but instead regulate it toward a balanced immune state. The reduction in IL-10, therefore, does not necessarily indicate heightened inflammation but rather suggests a dampened immune activation requiring less compensatory regulation, reflecting a reestablishment of immune balance. Another strong correlation with our

prior data is the differential expression of RANTES. In the SCI FM+EVs5 group, RANTES levels were significantly elevated (4-fold increase) compared to the SCI group, whereas the SCI FM+EVs10 group did not show this excessive upregulation. This dose-dependent effect of EVs on chemokine signaling and immune cell recruitment mirrors our earlier findings that higher doses of EVs were associated with improved functional recovery, likely due to better regulation of inflammatory cell infiltration. This also aligns with our electrophysiological data, where higher EV doses led to enhanced M-wave amplitudes and better motor conduction, suggesting a more controlled and neuroprotective immune response.

MSC-EVs have been shown to significantly inhibit the activation of the NLRP3 inflammasome and p38/MAPK signaling pathways, both of which are critical in triggering pro-inflammatory responses in mouse models of traumatic brain injury.[26] This inhibition led to reduced production of pro-inflammatory cytokines, including IL-1β and IL-6, thereby exerting an overall immunomodulatory effect.[27] Furthermore, animals treated with MSC-EVs exhibited improved cognitive and motor functions, reduced long-term inflammation, and decreased brain damage.[26,28] In a separate study involving a rodent model of SCI, administration of MSC-EVs was associated with a decrease in pro-inflammatory cytokines (IL-6, IL-1β), suppression of microglial reactivity,[29,30] and anti-apoptotic activity, leading to reduced neuroinflammation and enhanced recovery.[31] In addition, MSC-EVs administration significantly reduced TNF-α and IL-1β levels and activated autophagy, which further contributed to inflammation reduction and tissue regeneration.[32] Thus, our findings align with previous studies demonstrating that MSC-EVs modulate inflammatory processes by lowering pro-inflammatory cytokine levels and suppressing microglial activity in traumatic CNS injury models.

Taken together, these findings strongly correlate with our previous results,[12] reinforcing the idea that MSC-EVs fine-tune —rather than simply inhibit—the inflammatory response. This study focused on the chronic phase of SCI (60 dpi), a period marked by persistent inflammation and limited spontaneous regeneration. MSC-EVs may offer unique benefits in this context by promoting long-term immune modulation, potentially shifting microglia/macrophage populations toward anti-inflammatory states and supporting sustained tissue homeostasis. While no data from the acute or subacute stages were included, future studies with a temporal gradient (e.g., 7, 14, and 60 dpi) would allow a deeper understanding of cytokine dynamics and phase-specific therapeutic effects. Furthermore, although release kinetics were not directly assessed in this study, our findings support the idea that MSC-EVs delivered through FM can regulate pro- and anti-inflammatory cytokine levels in chronic SCI. This dynamic regulation likely underlies the functional improvements observed in our earlier study, including enhanced motor recovery (BBB scores), electrophysiological restoration (MEP, SSEP), and histological preservation of spinal cord tissue. Building upon our previous findings in rodent models, we extended our research to a porcine model of SCI to evaluate the translational potential of MSC-EVs.[33] In this feasibility study, we administered autologous MSC-EVs intrathecally during the subacute phase of SCI. The treatment led to partial restoration of locomotor function, which was associated with enhanced axonal remyelination and timely reperfusion of neural tissue. These outcomes further support the therapeutic promise of MSC-EVs in SCI recovery.

5. Conclusion

Our study demonstrated that the FM itself plays a crucial role in influencing inflammatory processes, promoting both pro-inflammatory and anti-inflammatory responses. This likely occurs through the activation of cells involved in tissue repair. In addition, the application of MSC-EVs, encapsulated in the FM, significantly altered the levels of both pro-inflammatory and anti-inflammatory cytokines in the chronic phase of SCI, a clinically relevant and understudied time point. The observed dose-dependent effects, particularly with the 10 μg MSC-EVs dose, suggest that this combination therapy may represent a novel approach for modulating inflammatory responses and promoting tissue regeneration after SCI.

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Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

The methods described herein were performed in accordance with the Declaration of Helsinki and approved by the local ethical committee of Kazan (Volga region) Federal University (No. 2, May 5, 2015).

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding author upon reasonable request.

Further disclosure

The paper has been uploaded to a preprint server (bioRxiv, doi: 10.1101/2024.10.07.616981).

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Appendix

Table A1. Multiplex analysis of cytokine levels (pg/mL) in spinal cord homogenates (Th8) from control (SCI and SCI FM) and experimental (SCI FM+EVs5 and SCI FM+EVs10) groups

SCI		G-SCF		GM-CSF	GRO/KC	IFN-γ	IL-10	IL-12p70	IL-13	IL-18	IL-1a	IL-1b	IL-2
Mean value		9.90		36.65	46.15	198.42	160.71	67.52	83.6	968.96	127.2	88.45	491.42
Standard deviation		11.37		18.20	17.68	167.21	147.75	45.12	67.25	154.43	45.73	22.71	236.89
SCI	**IL-4**	**IL-5**	**IL-6**	**IL-7**	**IL-17A**	**M-CSF**	**MCP-1**	**MIP-1α**	**MIP-3α**	**RANTES#**	**TNF-α**	**VEGF**	
Mean value	29.54	76.15	209.64	20.02	89.39	40.22	417.28	76.32	10.02	65.4	382.69	58.39	
Standard deviation	26.89	41.12	207.07	16.00	33.37	21.02	145.71	28.41	7.21	14.97	72.71	37.16	
SCI FM		**G-CSF***		**GM-CSF***	**GRO/KC**	**IFN-γ***	**IL-10****	**IL-12p70**	**IL-13**	**IL-18***	**IL-1a**	**IL-1b****	**IL-2***
Mean value		28.17		60.38	41.97	454.60	383.05	122.72	173.94	1229.89	115.34	133.09	955.11
Standard deviation		14.70		15.97	11.67	182.50	155.00	43.9	67.58	214.61	18.01	30.48	368.14
SCI FM	**IL-4**	**IL-5****	**IL-6***	**IL-7***	**IL-17A****	**M-CSF***	**MCP-1**	**MIP-1α**	**MIP-3α***	**RANTES**	**TNF-α**	**VEGF**	
Mean value	68.09	142.57	504.43	48.88	123.77	68.17	425.51	65.68	23.32	136.11	443.78	91.38	
Standard deviation	31.18	44.97	214.77	18.91	20.43	19.09	160.03	31.63	9.43	41.6	66.61	41.92	
SCI FM+EVs5		**G-SCF**		**GM-CSF**	**GRO/KC**	**IFN-γ**	**IL-10**	**IL-12p70**	**IL-13**	**IL-18**	**IL-1a**	**IL-1b**	**IL-2**
Mean value		15.73		51.06	39.15	299.97	267.65	96.98	112.00	1012.03	92.7	107.86	783.93
Standard deviation		8.8		16.61	14.59	161.49	129.06	56.27	59.22	387.98	44.66	44.21	368.62
SCI FM+EVs5	**IL-4**	**IL-5**	**IL-6**	**IL-7**	**IL-17A**	**M-CSF**	**MCP-1**	**MIP-1α**	**MIP-3α**	**RANTES**	**TNF-α**	**VEGF**	
Mean value	44.95	115.92	337.51	33.44	105.64	44.9	497.4	70.47	14.91	266.24	401.07	76.27	
Standard deviation	20.64	41.8	194.33	14.30	37.57	21.6	51.2	33.13	6.51	187.73	155.25	29.55	
SCI FM+EVs10		**G-SCF**		**GM-CSF**	**GRO/KC**	**IFN-γ**	**IL-10**	**IL-12p70**	**IL-13**	**IL-18**	**IL-1a**	**IL-1b**	**IL-2**
Mean value		9.99		38.09	33.75	204.49	156.77	61.94	89.95	1085.37	67.43	84.05	510.74
Standard deviation		11.97		26.77	11.93	200.25	168.55	55.11	80.9	193.01	49.62	32.49	322.05
SCI FM+EVs10	**IL-4**	**IL-5**	**IL-6**	**IL-7**	**IL-17A**	**M-CSF**	**MCP-1**	**MIP-1α**	**MIP-3α**	**RANTES**	**TNF-α**	**VEGF**	
Mean value	27.98	74.14	214.9	26.89	75.34	43.21	413.48	58.95	10.27	83.79	363.19	67.54	
Standard deviation	28.69	48.99	215.75	14.42	47.59	33.27	73.81	34.64	8.65	42.15	136.28	57.41	

Notes: *$p<0.05$ compared to SCI group; **$p<0.05$ compared to SCI and SCI FM+EVs10 groups; #$p<0.05$ compared to SCI FM and SCI FM+EVs5 groups.
Abbreviations: CSF: colony-stimulating factor; G-CSF: Granulocyte CSF; GM-CSF: Granulocyte–macrophage CSF; GRO/KC: Growth-related oncogene/keratinocyte-derived chemokine; IFN: Interferon; IL: Interleukin; M-CSF: macrophage CSF; MCP: Monocyte chemoattractant protein; MIP: Macrophage inflammatory protein; TNF: Tumor necrosis factor; VEGF: Vascular endothelial growth factor.

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