

HEALTH AND MEDICINE

Microplastics in the bloodstream can induce cerebral thrombosis by causing cell obstruction and lead to neurobehavioral abnormalities

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Human health is being threatened by environmental microplastic (MP) pollution. MPs were detected in the bloodstream and multiple tissues of humans, disrupting the regular physiological processes of organs. Nanoscale plastics can breach the blood-brain barrier, leading to neurotoxic effects. How MPs cause brain functional irregularities remains unclear. This work uses high-depth imaging techniques to investigate the MPs within the brain in vivo. We show that circulating MPs are phagocytosed and lead these cells to obstruction in the capillaries of the brain cortex. These blockages as thrombus formation cause reduced blood flow and neurological abnormalities in mice. Our data reveal a mechanism by which MPs disrupt tissue function indirectly through regulation of cell obstruction and interference with local blood circulation, rather than direct tissue penetration. This revelation offers a lens through which to comprehend the toxicological implications of MPs that invade the bloodstream.

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INTRODUCTION

Microplastics (MPs) are plastic particles with a diameter of less than 5 mm (1, 2). These particles originate from small plastic pellets produced for specific purposes, as well as from the degradation, weathering, and fragmentation of larger plastic products in the environment (3–5). MPs are ubiquitous worldwide, present in various environments ranging from oceans to land and from Antarctic ice to human settlements (4). Pollution from MPs is particularly notable in the oceans, where marine organisms such as fish, shellfish, and plankton ingest them, thereby introducing them into the human food chain (4–6). Substantial amounts of MPs have also been found in freshwater systems, including rivers, lakes, and reservoirs, allowing for contamination of human water sources. In addition, MP particles can be transported through the atmosphere and disseminated into the air, eventually entering the human respiratory system (7). Recent studies have indicated that MPs can directly enter the human bloodstream through the use of plastic medical supplies (8).

MPs have been found in human feces and various tissues, including the liver, kidney, placenta, and blood (9–11). Accumulation of MPs in organisms can result in tissue dysfunctions and chronic diseases such as respiratory diseases, immune system disorders, chronic inflammation, endocrine gland effects leading to hormone imbalance, and metabolic dysfunctions (12–15). In particular, the presence of MPs in the bloodstream poses a substantial health challenge. As the blood circulates, these MPs may be carried to any organ, especially the distal branch vessels. Studies have shown that blood MPs can lead to acute cardiovascular diseases (16–17). Furthermore, in a study, patients with carotid artery plaque in which MPs and nanoplastics (NPs) were detected had a higher risk of a composite of myocardial infarction,

stroke, or death from any cause in 34 months (18). These potential threats can be life-threatening compared to the chronic diseases caused by MPs. In addition, MPs can cause brain dysfunction. Nano-sized plastic particles can penetrate the blood-brain barrier (BBB) and enter brain tissue (19, 20). The interaction between NPs and α -synuclein fibrils can exacerbate the spread of α -synuclein pathology in vulnerable brain regions, potentially triggering or worsening conditions such as Parkinson's disease and other neurologically related dementia diseases (21). However, how micron-sized plastics affect brain function remains unclear. Even several studies have proved that treatment with MPs affects behavior and induces phenotypes such as anxiety in mice (22, 23). It is supposed that micron-sized plastics break down into nanosized plastic particles in the body and then enter the brain to exert their effects. It has also been suggested that the impact of MPs on the brain may be mediated through their effects on peripheral tissues, including the modulation of immune inflammation and glycolipid metabolism (24–27). What is the mode of MPs affecting the brain and the mechanisms that they exert their effects remain uncertain.

The development and innovative application of previously unknown research techniques often open new avenues, providing researchers with fresh perspectives and facilitating a deeper understanding of scientific principles. In this study, we applied miniature two-photon microscopy (mTPM) and imaged MPs in the mouse brain in vivo while the animal was awake. With the high-depth imaging capability, we observed MPs in the blood vessels of the mouse cerebral cortex. We tracked the high-speed movement of MP particles in the blood vessels, revealing a mechanism by which MPs can induce brain dysfunction and neurological impairment.

RESULTS

Blood vessels exhibit strong contrast and weak fluorescence signals in the imaging of the brain cortex in living mice

To examine the effects of MP particles in the brain, we chose to first image brain blood vessels. We hypothesized that, even if MPs are first broken down into NPs to pass through the BBB and affect neuron function, it is needed for NPs to arrive at the local brain vessel and

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try to cross the BBB; thus, we can catch the process by the vessel imaging. Moreover, an imaging strategy incorporating fluorescent MP microspheres can help us to test this hypothesis.

To conduct *in vivo* cerebral vascular imaging in mice, we used an appropriate imaging system (fig. S1, A and B) (28). Surgical intervention was necessary to attach the mouse head assembly onto the microscope lens (Fig. 1A). Following the surgical procedure, the vascular network of the cerebral cortical tissue could be observed through the imaging window in the head, as depicted in the field of view (FOV) (Fig. 1B) under bright-field imaging. The expression of fluorescent probes in neurons via adeno-associated virus served as a background signal for vascular imaging, enhancing the contrast of blood vessels. After single-photon fluorescence excitation, an optimal imaging position was identified within the FOV. Both blood

vessels (BV) and blood flow were visible within FOV2 and FOV3 (Fig. 1B; refer to movie S1). Selecting an ideal imaging area, fluorescence imaging of deep brain tissue was conducted using two-photon excitation within FOV4 (Fig. 1B).

There was no fluorescent signal in the blood, and the vessels and surrounding tissues in the imaging FOV showed high signal contrast. Converting the image to three-dimensional (3D) for signal presentation, we could visualize the vessel channel more clearly (Fig. 1C). The regions of interest (ROIs) were outlined in the image, and the fluorescence intensity was calculated. The fluorescence values for the linear ROIs a# and d# exhibited a significant decrease in fluorescence intensity at the blood vessels (Fig. 1D). In contrast, the long-term fluorescence values for ROIs 1# and 4# indicated stable fluctuations around a certain value, suggesting that

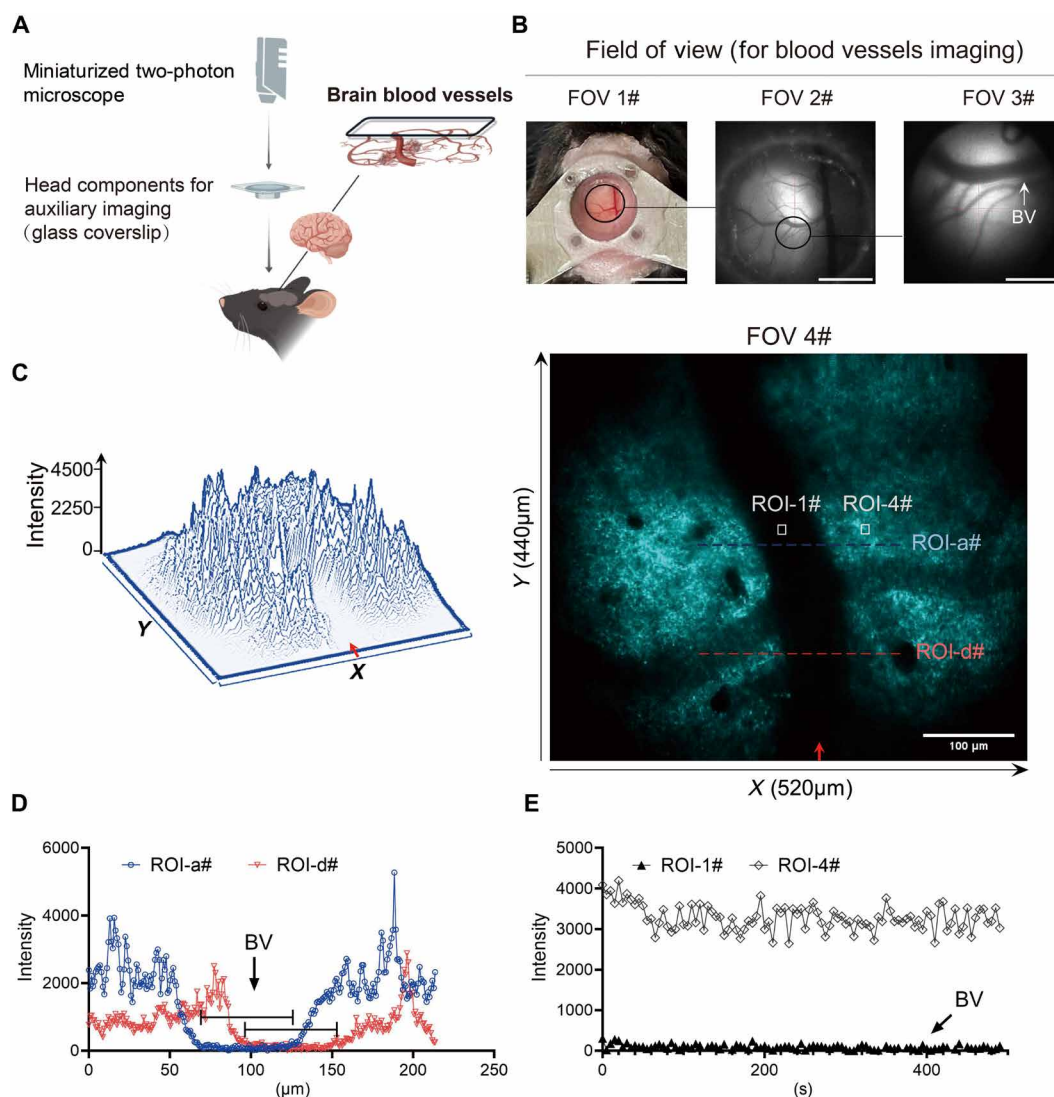


Fig. 1. Blood vessels exhibit strong contrast and weak fluorescence signals in brain cortex imaging by using two-photon microscopy in living mice. (A) Schematic imaging representation by miniaturized two-photon microscopy to cortical blood vessels (BV). Created using FigDraw.com. (B) Fields of view (FOVs) of the blood vessels: FOV1#, bright field (scale bar, 1 cm); FOV2#, 488-nm single-photon excitation (scale bar, 250 mm); FOV3#, 488-nm single-photon excitation (scale bar, 100 mm); and FOV4#, 920-nm two-photon excitation (scale bar, 100 μm, a red arrow indicates the location of blood vessel). (C) Three-dimensional (3D) mapping of fluorescence signals within the FOV 4#. (D) Fluorescence signal detections for regions of interest (ROIs) a# and d#. (E) The fluorescence signal is recorded over a long period for fluorescence signal detections for ROIs 1# and 4#.

blood flow did not induce significant or irreversible changes in the signal (Fig. 1E and fig. S1, C to E). Together, these experiments suggest that the imaging system is suitable for targeting fluorescence signal detection in blood vessels of the brain in vivo.

MPs were detected in the imaging of cerebral blood vessels in vivo

Using this imaging system, we attempted to visualize fluorescent MP particles that may be present in the bloodstream. The typical blood flow in mice ranges from 2.5 to 18 cm/s, slowing down significantly in capillaries to a few hundred micrometers per second (29). Because of the limitations of the imaging FOV, to detect MP particles moving with the blood, imaging frequencies below 1 Hz are required. After selecting a clear FOV for vascular imaging, mice were gavaged with water containing fluorescently labeled MP particles (Fig. 2, A and B). To increase the likelihood of capturing MPs images, we aimed to maximize the effective imaging time. Therefore, imaging was initiated 1 hour after MP treatment (Fig. 2B).

We first observed MPs moving within the bloodstream 2 hours and 20 min after treating mice (Fig. 2C; refer to movie S2). The movement of the MP resembled a lightning bolt in a dark vascular region. The trajectory of the MP appeared shuttle shaped (Fig. 2C). In particular, one MP track observed at 3 hours and 4 min resembled a comet with a trailing tail. The imaging time for one frame is 208 ms, and the entire MP trajectory was captured within this frame time (Fig. 2D). At the site of MP emergence, there is a significant change in fluorescence value, as shown in Fig. 2D. To simplify and illustrate this phenomenon in the text, we have coined the term “microplastic flash (MP-Flash)” to describe the intravascular movement of MP particles. In our study with five mice, MP-Flash was observed in four of them (Fig. 2E). All four mice exhibited MP-flashes 2 hours after water treatment. Among the four mice showing MP-Flash, it was detected once in one mouse (Fig. 2E). The median time for the appearance of MP-Flash after drinking was 191 min (Fig. 2F). The trajectory lengths of the MP-Flash were all less than 330 μm (Fig. 2G), and the entire trajectory was captured in a single imaging frame, lasting less than 208 ms. The median length of the MP-Flash trajectory was 57.5 μm (Fig. 2H and fig. S2A). There is no linear correlation between the appearance time of these MP-Flashes and their track lengths (fig. S2B). In addition, the median running speed of MP particles during MP-Flash was calculated to be 321 $\mu\text{m/s}$ (Fig. 2I), comparable to the blood flow rate.

However, we could not guarantee that the vessels imaged in each mouse were of the same size type, potentially leading to differences in MP-Flash velocity and trajectory length (Fig. 2G). In summary, through millisecond imaging of deep tissue vessels, we successfully detected MP-Flash in animals and calculated several basic characteristic indices. These results show an effective method for detecting MPs moving within the cerebral vasculature of living animals.

Circulating MPs are phagocytosed by cells in the blood

After 3 hours of fluorescent MP treatment, we found that cells labeled with fluorescent signals were observed in the bloodstream (Fig. 3A and fig. S3A; refer to movie S3). However, in the absence of fluorescent MP treatment, these signals were never detected, even with a longer imaging duration (>3 hours). The fluorescence intensity of these cells was comparable to that of the MP-Flash signal, which was significantly higher than the fluorescence signal from probe-expressed neuronal cells (Fig. 3B). Thus, we hypothesized

that fluorescent MPs that enter the bloodstream are phagocytosed by cells, which are labeled with a fluorescent signal. To test this hypothesis, we directly injected MPs into the bloodstream of mice via intravenous injection (Fig. 3C). The MP-Flash signal was observed in the cerebral cortical blood vessels within minutes following intravenous injection, as compared to gavage administration (Fig. 3D). There was no significant difference in the trajectory length of the MP-Flash signals observed (Fig. 3E). Unexpectedly, the fluorescence-labeled cells could be detected ~10 min after intravenous injection (Fig. 3F). We compared the timing of MP-Flash appearance and the fluorescence-labeled cells in each experiment, and we found that the fluorescence cell typically appeared within 6 min of observing the MP-Flash (Fig. 3F). Moreover, no similar fluorescence cells were observed in the blood under non-treated conditions. Collectively, fluorescent MPs led to the emergence of fluorescence-labeled cells in the blood, suggesting the MPs that enter the bloodstream are phagocytosed by cells.

Next, we tracked and observed these cells. We named them MP-labeled cells (MPL-Cells). Using ImageJ, we calculated the diameters of these cells, which were around 21 μm (Fig. 3G and fig. S3B). We further discovered that the movement of MPL-Cells in blood vessels was not simply synchronized with the blood flow. In certain tests, we noticed that MPL-Cells would become trapped in specific locations within the blood vessel and remain there for an extended period. The movement trajectory of an MPL-Cell in the cortical vasculature is depicted in Fig. 3H (fig. S3C). It took nearly 10 min for this MPL-Cell to enter and leave the imaging FOV, however, showing nonuniform motion with only a few seconds spent in most segments of the trajectory, as indicated in motion trajectories 1 and 3 (Fig. 3I). Motion trajectory 2 consumed most of the time during the entire motion process, despite covering the shortest distance. This MPL-Cell encountered a nearly 90° turn in motion trajectory 2, which might be the reason leading to its obstruction. By analyzing similar events, we defined an obstructed cell as one that remained trapped at a position in the vessels for over 2 min. Some MPL-Cells eventually exited the imaging FOV following a period of obstruction due to blood flow (Fig. 3J; refer to movie S4). The trajectories of these cells can be categorized into three stages, with some being directly obstructed without progressing to stage 1 and later leaving after a period of blockage. Notably, we observed that around 18% of the cells remained blocked in the same position until the end of the imaging session (Fig. 3K and fig. S3D). Furthermore, we did not observe MPs attaching to the vessel wall or crossing the vessel wall into the brain tissue.

Fluorescent MPL-Cells were sorted and characterized

Next, we tried to sort the MPL-Cells (Fig. 4A). Mouse blood was collected from the heart 20 min after injecting the fluorescent MPs. The isolated cells were then analyzed by flow cytometry, and a distinct population of fluorescein isothiocyanate (FITC)-positive cells was observed in the injected group compared to that in the untreated control group (Fig. 4B and fig. S4, A and B). All five mice that received injections showed clear FITC-positive cells (Fig. 4C). Immune cells are known to have a strong phagocytic ability toward exogenous pathogens and foreign substances (30). Our findings revealed that ~94% of the FITC-positive cells in the treatment group were positive for the cell surface antibody CD45, indicating that they were immune cells (Fig. 4D and fig. S4C). To identify the immune cell type, we further labeled the cells with antibodies F4/80 and Ly6G/C (Fig. 4E

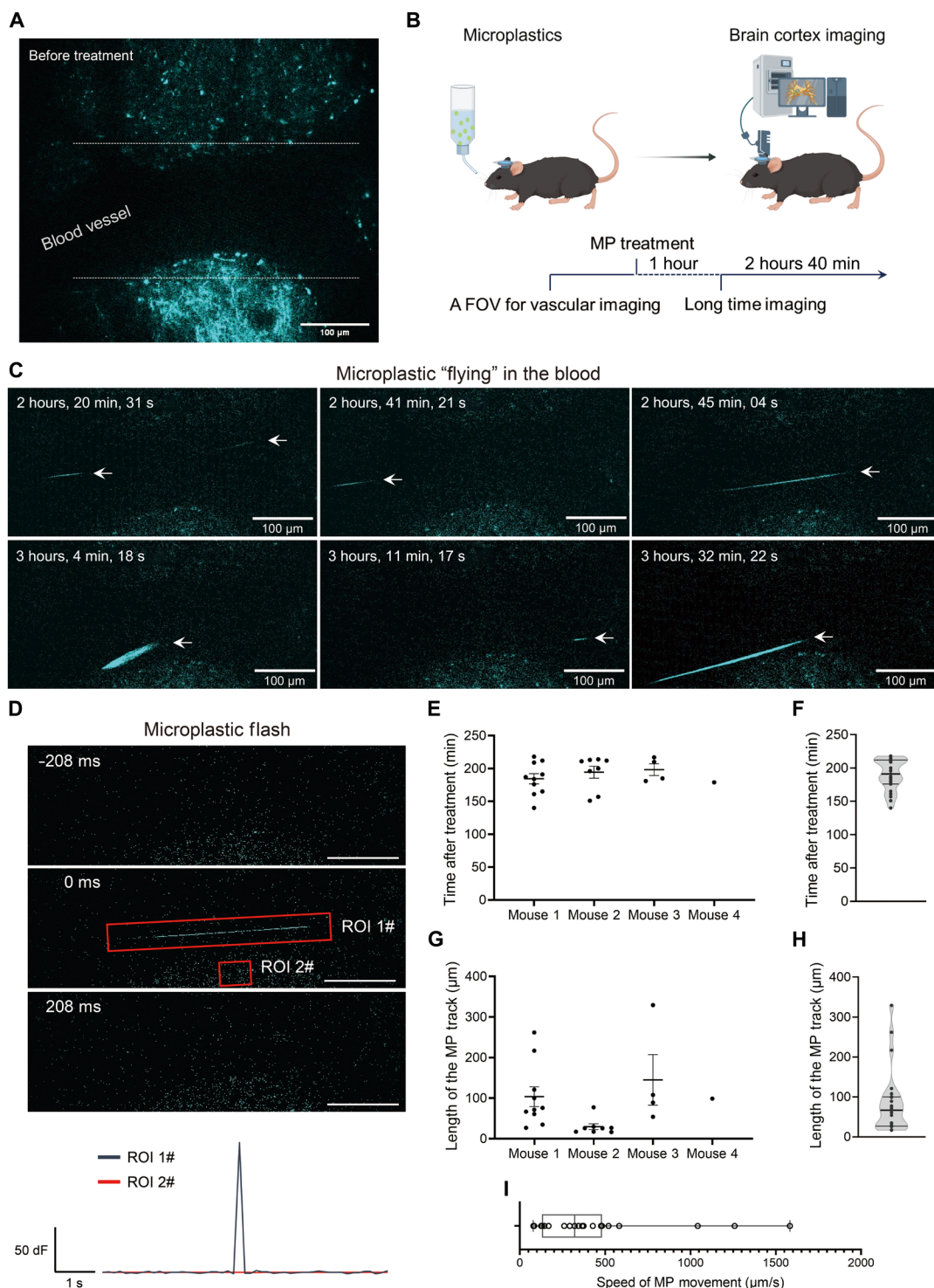


Fig. 2. MPs were detected in the imaging of cerebral blood vessels in vivo. (A) A suitable FOV for vascular imaging. (B) Schematic representation of the treatment and imaging design of the mice. Created using FigDraw.com. (C) Captured MP-Flash images; this FOV is the rectangular FOV framed by the dashed line in (A) (scale bar, 100 μ m). (D) The emergence of an MP-Flash is demonstrated; ROI 1# represents the MP-Flash, and the peak of wave that exhibits a fluorescent signal is present; ROI 2# represents the background control, no fluctuation in fluorescence value. (E and F) Comparison of the time of MP-Flash appearance ($n = 10$ from mouse 1, $n = 8$ from mouse 2, $n = 4$ from mouse 3, and $n = 1$ from mouse 4) (E), and the median time of the MP-Flash occurrence (F). (G and H) Comparison of the length of the MP-Flash track (G), and the median length of the MP-Flash track (H). (I) Calculated MP-Flash running speed ($n = 23$ from four mice). Data are represented as means $\pm$ SEM.

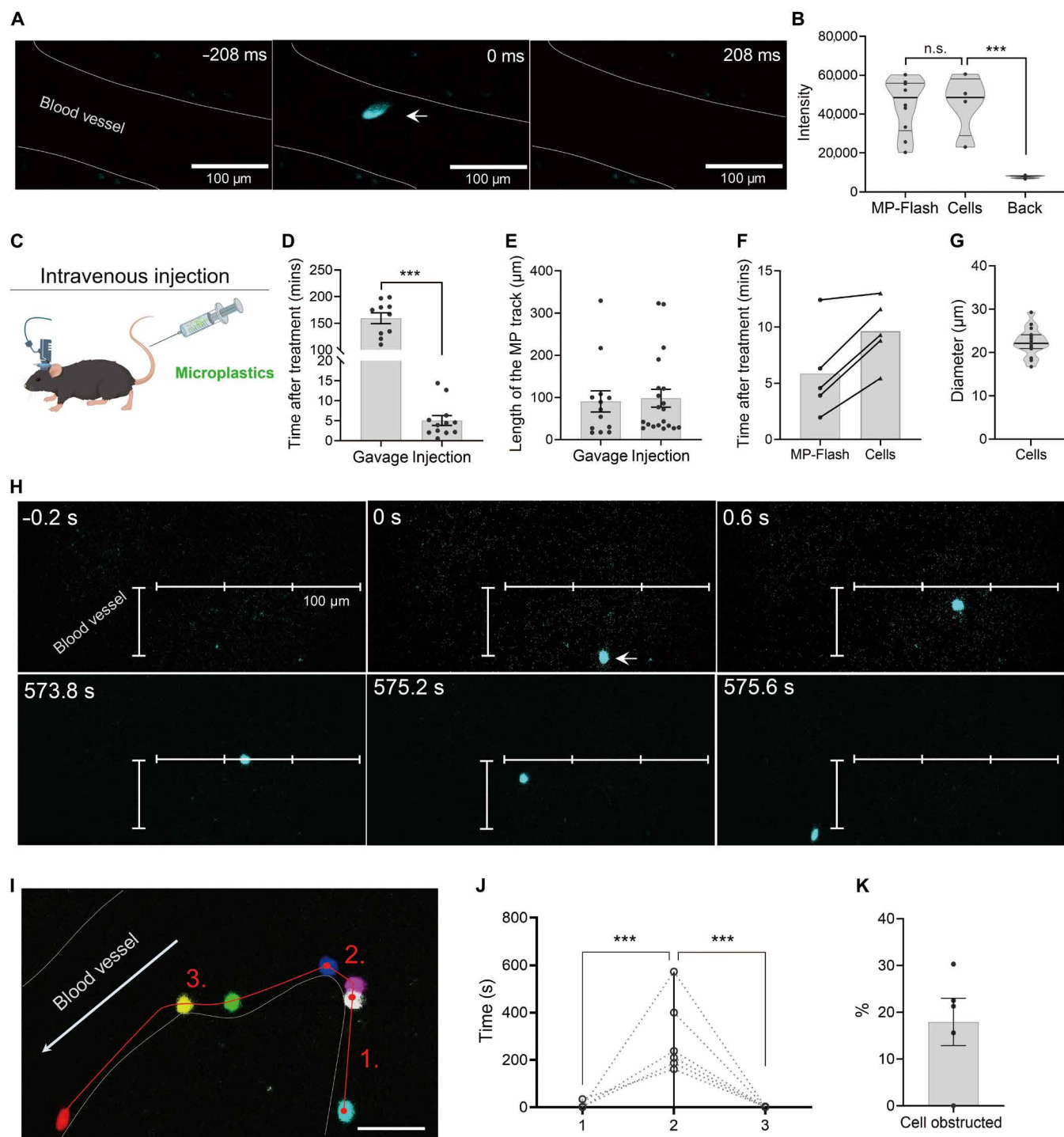


Fig. 3. Circulating MPs are phagocytosed by cells in the blood. (A) A representative fluorescent signal labeled cell. (B) Quantitative comparison of fluorescence signals ($n = 10$ MP-Flash, $n = 4$ fluorescence signal labeled cells, and $n = 10$ background ROIs) (three independent replicated experiments verified the result). (C) Schematic representation of the intravenous injection in mice. Created using FigDraw.com. (D and E) Comparison of MP-Flash emergence times ($n = 10$ to 12 from three mice) (D) and lengths ($n = 13$ to 20 from three mice) (E) between the two treatments. (F) Comparison of the time of appearance of MP-Flash and the time of appearance of fluorescence-labeled cells after injection ($n = 5$ experiments from five mice). (G) Diameter statistics for fluorescence-labeled cells ($n = 15$ from four mice). (H) A trajectory of a fluorescence-labeled cell and the corresponding time. (I) The movement trajectory can be labeled as three motion processes. (J) Statistical analysis of the time taken by the three processes for equal events ($n = 6$ events from four mice). (K) Percentage of labeled cells that are consistently obstructed ($n = 5$ FOVs from five mice). Data are represented as means $\pm$ SEM. *** $P < 0.001$. n.s., not significant.

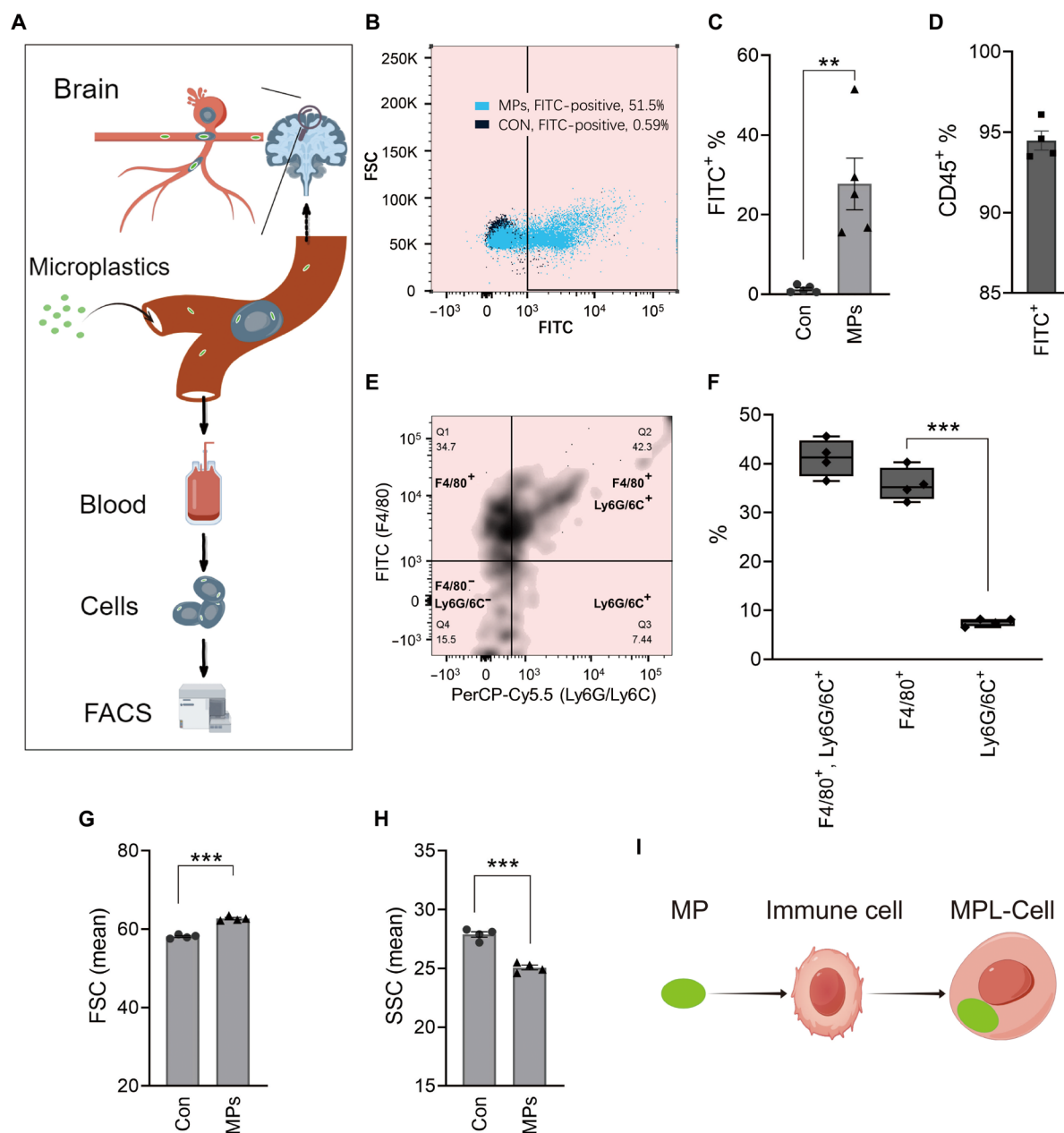


Fig. 4. Fluorescent MPL-Cells were sorted and characterized. (A) Schematic representation of the MPL-Cells in cerebral vessels, isolation, and characterization. Created using FigDraw.com. (B) Representative fluorescence-activated cell sorting (FACS) analysis plots for fluorescein isothiocyanate (FITC)-positive cells. (C) Quantification of FITC-positive cells ($n = 5$ mice for each group, more than 1×10^5 cells were counted per sample). (D) Percentage of CD45-positive cells among FITC-positive cells in MP-treated mice ($n = 4$ mice). (E and F) FACS analysis plots (E) and quantification (F) for the F4/80-positive and Ly6G/Ly6C-positive cells ($n = 4$ mice). (G and H) Analysis of the FSC (G) and SSC (H) in the MP-labeled neutrophils and control. (I) Illustration of the effects of phagocytosis of plastic microspheres on cell morphology. Data are represented as means $\pm$ SEM. ** $P < 0.01$ and *** $P < 0.001$.

and fig. S4, D and E). The results indicated that the majority of the immune cells exhibited double positivity for F4/80 and Ly6G/C (~41%) or single positivity for F4/80 (~35%) (Fig. 4F). This suggested that the cells involved in phagocytosis of MP particles were predominantly neutrophils (F4/80⁺ and Ly6G⁺) and macrophages (F4/80⁺ and Ly6G⁻), supporting our hypothesis as neutrophils and macrophages are known to have strong phagocytic capabilities. We compared neutrophils that had phagocytosed MPs with unphagocytosed controls, showing a significant increase in forward scatter (FSC) and a

decrease in side scatter (SSC) (Fig. 4, G and H). These results indicate that the engulfment of MPs affects cell size and surface status, potentially contributing to MPL-Cell obstructions (Fig. 4I).

Obstructed MPL-Cells induced long-time blockages in the cerebral blood vessels

The imaging data revealed that some MPL-Cells would exit the imaging FOV after being obstructed in the cerebral vessels for a period of time. However, there were still MPL-Cells that remained obstructed

in the blood vessels even after an extended imaging duration. We observed three MPL-Cells still present within the FOV following nearly 2.5 hours of imaging (Fig. 5A). These three cells exhibited distinct morphological characteristics, displaying both regular oval and ruffled morphology. To obtain clearer observations of the obstructed cells, we used mice without labeled neurons to minimize background

signals and exclusively capture the fluorescence signal of the MPL-Cells. Subsequently, we observed that MPL-Cells exhibited diverse morphological features due to their nonalignment in the same horizontal imaging plane. As depicted in Fig. 5B, we captured the imaging plane at two different depths, I and II. Altering the imaging depth resulted in the transformation of cell I-a from ellipsoidal to ruffled

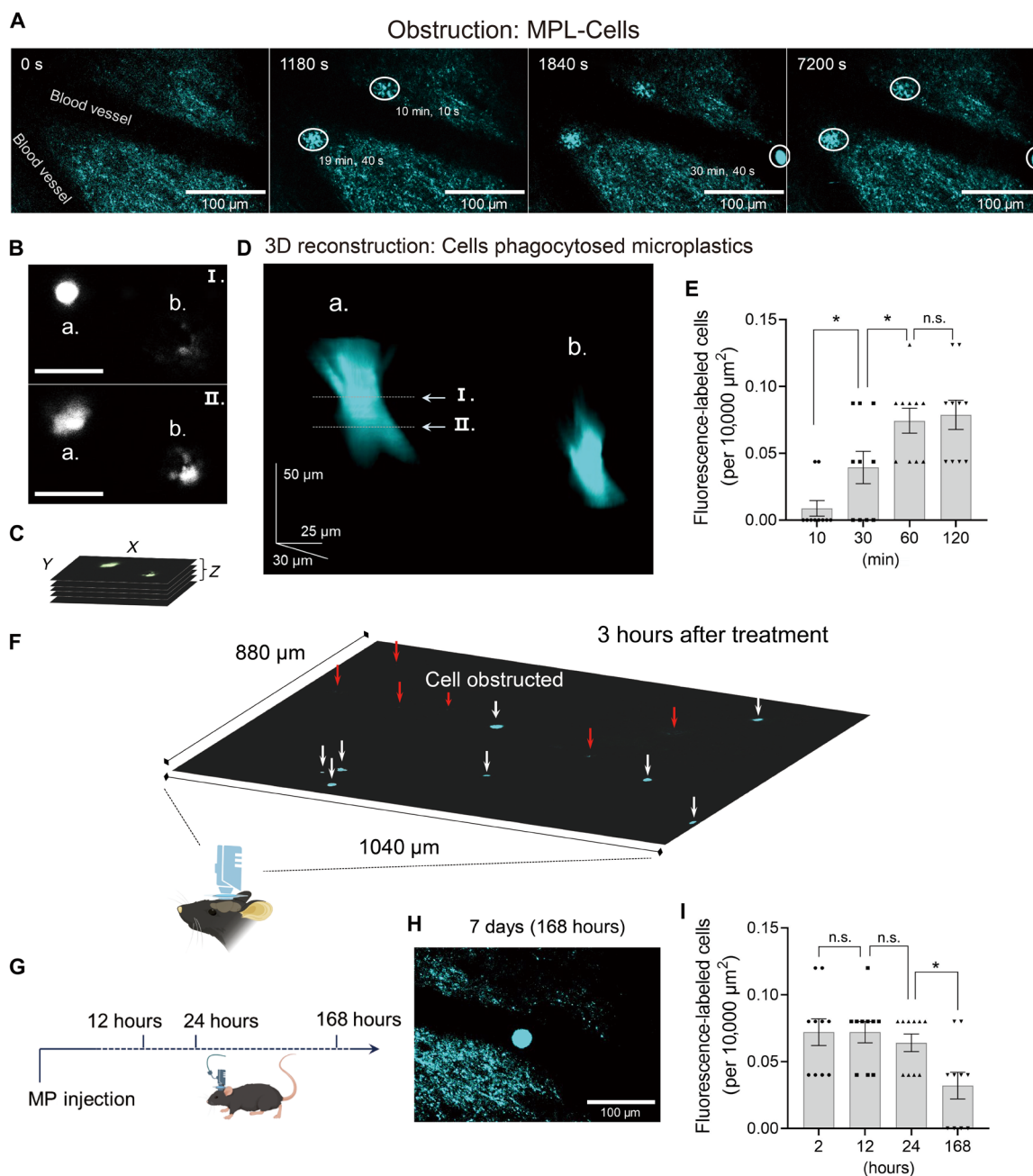


Fig. 5. Obstructed MPL-Cells induced long-time blockages in the cerebral blood vessels. (A) Representative MPL-Cells, time points in which cells appear were marked on the graph, and time 0 represents the imaging start. (B to D) Imaging of MPL-Cells sectioned at different depths (B); z-stack imaging of the MPL-Cells (C); reconstruction of MPL-Cells 3D images, two sections, I and II, of cell correspond to the imaged sections in (B). (D). (E) The density of MPL-Cells within each two-photon imaging FOV was calculated and analyzed at different time points after treatment ($n = 10$ FOVs from four mice per time point). (F) Showing MPL-Cell distribution at larger FOV by image stitching, at 3 hours after MP injection. (G) Long-time imaging arrangements. Created using FigDraw.com. (H) An MPL-Cell that has been obstructed in a vessel for 7 days. (I) The density of MPL-Cells in the brain cortex at the indicated time points ($n = 10$ FOVs from four mice per time point). Data are represented as means $\pm$ SEM. * $P < 0.05$.

II-a, while cell I-b changed from ruffled to ellipsoidal II-b. To generate 3D images of the cells, we conducted z-stack imaging (Fig. 5C and fig. S5A).

We observed that MPL-Cells exhibited a vertical morphology, appearing as if they were elongated and inserted into blood vessels (Fig. 5D; refer to movie S5). Different imaging depths revealed varying cross sections of the longitudinal depth of the cells, as depicted in Fig. 5B (I and II). We speculate that MPL-Cells may be mostly obstructed at the junction of blood vessels extending from the deep brain toward the cortex with laterally distributed blood vessels within the cortex, where there would be greater vascular curvature (31).

Next, the distribution of MPL-Cell blockage density in the cortex was quantitatively analyzed at different time points after injection (fig. S5B). The number of blocked cells was significantly higher at 1 hour after treatment compared to half an hour (Fig. 5E). The obstruction density remained high at 2 hours after treatment, with no significant difference to 1 hour. By using a post-imaging image stitching method, the distribution of MPL-Cells across a large-scale range in the cerebral cortex is presented (Fig. 5F). Upon analysis of the numerous cells, it was observed that these cells predominantly obstructed in the vessels that are perpendicular to the cortex, as depicted in Fig. 5B.

Moreover, how long will the MPL-Cells, which are obstructed in blood vessels, last before being cleared? The brain contains a large number of capillaries, and the blockage of these cells may affect

blood flow and potentially result in neurological dysfunction (32–34). We conducted a 1-week assay on treated mice (Fig. 5G), and, unexpectedly, 7 days after treatment, the cells were still not cleared (Fig. 5H), although the density of blocked cells had been significantly reduced (Fig. 5I).

Cell obstruction is influenced by the size of the plastic particles

Furthermore, the question arose as to whether the mechanism of MPL-Cells blocking blood vessels is specific to micrometer-scale plastics. Does the size of the plastic particles affect the obstruction of these labeled cells? Subsequently, mice were treated similarly with 2- and 0.08- μm diameter fluorescent plastic particles, we detected these fluorescent plastic particles within the blood vessels and, subsequently, observed cells labeled by them (Fig. 6A). The area of detectable fluorescent signals is smaller in cells labeled with 2- μm plastic particles compared to those labeled with 5 μm . The morphology of the signal in the 2- μm plastic particle-labeled cells does not exhibit a fully spherical shape; instead, it appears as a stronger signal at the core, surrounded by a weaker ring of dispersed signals (Fig. 6A; refer to movie S6). In contrast, the morphology of the signals in cells labeled with 0.08- μm plastic particles is much less distinct, with no obvious core visible and a weaker signal (Fig. 6A; refer to movie S7). Two hours after exposure to plastic particles, we measured the

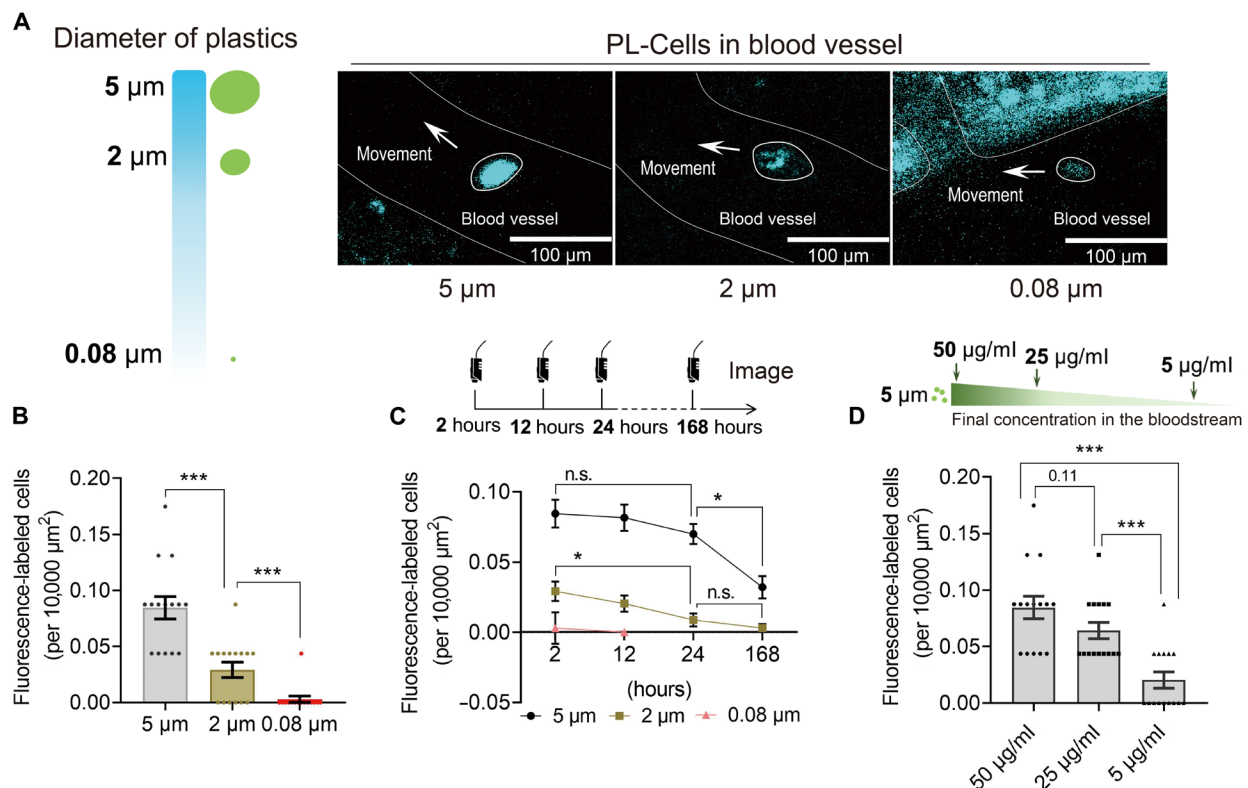


Fig. 6. Cell obstruction is influenced by the size of the plastic particles. (A) Exposure experiments were conducted on mice using plastic particles of varying sizes, and images of cells labeled with fluorescent plastics of different sizes were captured during the imaging of blood vessels. (B) The density of plastic-labeled cells (PL-Cells) within each FOV was calculated and analyzed. Data collected at 2 hours after treatment ($n = 15$ FOVs from four mice for the 5- μm group and $n = 15$ FOVs from two mice for the 5- μm and 0.08- μm groups). (C) PL-Cell densities at different time points after exposure were comparatively analyzed ($n = 15$ FOVs from four mice for the 5- μm group and $n = 15$ FOVs from two mice for the 5- μm and 0.08- μm groups). (D) A comparative analysis of PL-Cell density was performed following exposure to different concentrations of 5 μm MPs ($n = 15$ FOVs from four mice for group at 50 $\mu\text{g/ml}$ and $n = 15$ FOVs from two mice for groups at 25 and 5 $\mu\text{g/ml}$). Data are represented as means $\pm$ SEM. * $P < 0.05$ and *** $P < 0.001$.

extent of the cell obstruction in the brain. Unexpectedly, the levels of PL-Cell obstruction in the cerebral vasculature of mice exposed to 2- μm plastic particles were significantly lower. Cells labeled with 2- μm particles demonstrated an obstruction level that was over 50% lower than that of cells labeled with 5- μm particles (Fig. 6B). Furthermore, the obstruction level in cells labeled with 0.08- μm plastic particles was even lower, as only one obstructed cell was observed across 15 FOVs in two mice (Fig. 6B). Compared to the 5- μm group, there was a notable reduction in the obstruction level in the 2- μm group within 24 hours (Fig. 6C). These findings indicated that not only did the cells labeled by 2- μm plastic particles experience lower levels of obstruction, but the obstructed cells were also more readily cleared. Consistent with that, cells labeled by 0.08- μm plastic particles completely vanished within 12 hours (Fig. 6C). These findings suggest that plastic particle size is a crucial factor in regulating MPL-Cell obstruction, when particles are no larger than 5 μm in diameter, a smaller particle size corresponds to a lower level of obstruction.

Exposure concentration is a crucial indicator for evaluating the biotoxicity of MPs. To enhance comparability with actual human exposure level (12 $\mu\text{g}/\text{ml}$ in the blood) (8), we tested various concentrations using 5- μm MPs. The obstruction level of MPL-Cells was not significantly affected when the exposure concentration was halved (Fig. 6D), suggesting that the original exposure concentration may have reached a saturation point that leads to MPL-Cell

obstruction. However, upon reducing the final concentration to 5 $\mu\text{g}/\text{ml}$, a marked decline in cell obstruction levels was observed (Fig. 6D). Notably, cell obstructions within the cerebral vasculature can be observed even in the lowest-concentration (5 $\mu\text{g}/\text{ml}$) group of MPs.

MPL-Cell obstructions as thrombus formation inhibit intracerebrovascular blood perfusion

To investigate the impact of MPL-Cell obstruction in blood vessels on blood flow, we used laser speckle contrast imaging (LSCI) to monitor blood flow in the mouse brain. We observed changes in blood flow at 30 ROIs in the cerebral cortical vessels at various time points after injection (Fig. 7A and fig. S6). The LSCI results indicated that treatment with MPs led to a reduction in blood perfusion levels in the vessels (Fig. 7B), particularly notable at 30 min after treatment, with significantly lower perfusion levels compared to 10 min.

Subsequently, we classified the vessels based on their flow levels to further analyze the impact of MPs on vessels with different flow rates. The ROIs were divided into three groups on the basis of the measured blood perfusion levels: perfusion units (PU) greater than 500, PU greater than 400 but less than 500, and PU less than 400. Among these, the ROIs with PU greater than 500 exhibited no significant changes over the course of measurement (Fig. 7C). Conversely, the other two groups showed a noticeable decrease in blood perfusion levels starting at 10 min after treatment (Fig. 7, D and E),

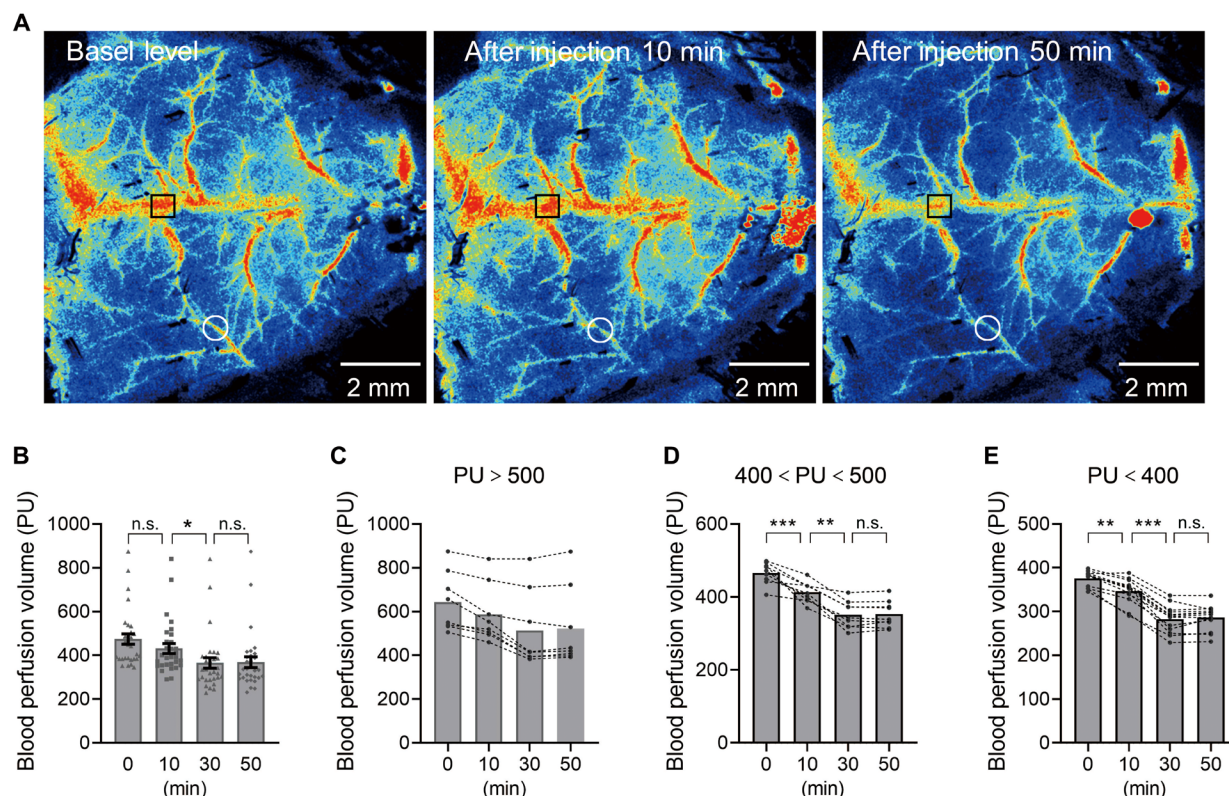


Fig. 7. MPL-Cell obstructions as thrombus formation inhibit intracerebrovascular blood perfusion. (A) Representative laser speckle contrast imaging (LSCI) images at indicated time points after treatment. A black line square identifies an ROI location with perfusion units (PU) > 500, and a white circle identifies an ROI location with PU < 400. (B) Quantitative statistical analysis of ROIs ($n = 30$). (C to E) Analysis of ROIs with PU > 500 ($n = 8$), ROIs with $400 < \text{PU} < 500$ ($n = 9$), and ROIs with PU < 400 ($n = 13$). Three independent replicated experiments verified the results. Data are represented as means $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

reaching their lowest point at 30 min. These findings suggest that MPL-Cell obstruction in the vessels affects blood perfusion, particularly affecting smaller vessels with lower blood flow.

Suppressed blood flow in the brain causes neurobehavioral disorders

Vascular embolism resulted in insufficient blood supply to the brain tissue, leading to disturbances in neural activity and cognitive impairment in mice (35, 36). We investigated whether obstruction of MPL-Cells caused behavioral changes in mice (Fig. 8A). The open-field experiment is a widely used method for assessing the exploratory behavior of rodents, particularly in the context of anxiety disorders and other neurological and psychiatric conditions. Mice were assessed in the open field 6 hours after injection (Fig. 8B). The total distance and speed of movement of MP-treated mice were significantly lower compared to those in the control group (Fig. 8, C and D). No significant difference was observed in the number of entries into the center area (Fig. 8E). In the Y-maze test for assessing working memory (Fig. 8F), the total distance traveled by MP-treated mice remained lower than that in the control group (Fig. 8G), with a significant reduction in spatial memory (Fig. 8H). These findings also suggest that MPs potentially inhibit motor abilities in mice. To investigate this further, a rotarod test was conducted to evaluate motor coordination. MP-treated mice exhibited a significant decrease in latency time (Fig. 8I). Similarly, in the rod-hanging test, MP-treated mice showed a significant decrease in hanging time at 1 and 3 days after injection, indicating reduced coordination and endurance. However, this difference was no longer significant on day 7 (Fig. 8J). The effects of MPs on mouse locomotion may influence performance in open field and Y-maze tests. These findings indicate that mice display multifaceted abnormalities in neurobehavioral regulation, resembling depressive states associated with disrupted cerebral blood flow (37, 38). By day 28 after MP injection, the rotarod test showed no significant difference (Fig. 8K), suggesting that the impairment in behavioral abilities caused by MPL-Cell blockage and altered blood perfusion was restored by 28 days. In addition, MPs were found to induce weight loss in mice (Fig. 8L), potentially due to changes in feeding behavior resulting from altered locomotor abilities. Last, at 28 days after injection, the recovery of MPL-Cell obstruction in the cerebral vasculature was observed, with a significantly lower density compared to 7 days after injection (Fig. 8M). These results align with the behavioral experiments conducted in mice at 28 days after injection.

DISCUSSION

For an extended period, the application of advanced technology—through long-distance (e.g., astronomical sciences), large-scale (e.g., earth sciences), or microworld (e.g., life sciences) imaging—has served as a crucial method for scientific researchers to conduct explorations and summarize the underlying principles governing the various phenomena. We used fluorescent MPs and *in vivo* imaging to detect the MPs in the circulatory system of an organism. The MP-Flash was captured in clear trails in their entirety. The establishment of this imaging system enables the visualization of MPs at the *in vivo* level within the organism while simultaneously providing researchers with a powerful tool for studying the role of MPs in the organism. Using this imaging system, our results demonstrate that immune cells phagocytosed the MPs in the bloodstream, termed MPL-Cells (fig. S7). We captured images of the MPL-Cells in motion and found that these cells can

obstruct cerebral blood vessels. By combining multilayer scanning imaging with 3D reconstruction techniques, we obtained clear images of the blocked MPL-Cells (Fig. 5, B to D). Long-duration tracking imaging showed the MPL-Cells present difficulties in clearance for a prolonged period of at least 1 week. The obstruction notably hindered blood flow in the vessels, resulting in impaired neurological function in the mice. The direct observation of MPL-Cells provides a mechanistic explanation for MP-induced neurological dysfunction.

Nevertheless, the application of this technology remains in its early stages, and there are still several deficiencies in data collection and analysis. Visual documentation of MPs in circulating blood was obtained (Fig. 2C). Using a millisecond-scale imaging technique, the movement trajectory of the MP-Flash was captured within 208 ms, enabling the calculation of the motion velocity of the MP (Fig. 2I). Nevertheless, it is important to acknowledge the constraints associated with this calculation. First, there is uncertainty regarding whether the motion of the MP-Flash falls entirely within the imaging plane, potentially leading to an underestimation of the trajectory length used for calculation. In addition, despite the utilization of high-speed bidirectional line scan mode for image acquisition, the total imaging time for the entire FOV upon completion of the scan was 208 ms, suggesting that the actual motion time of the MP-Flash may be shorter. These factors may have biased our calculations low. Moreover, the inherent physical characteristics (diameter, polystyrene material, and spherical) of the MP particles used in our study present constraints on our imaging techniques and computations, potentially limiting their generalizability to other varieties of MPs.

Our findings suggest that the phagocytosis of MPs is mainly by immune cells in the blood. Nevertheless, the underlying reasons for immune cells obstructing the vasculature after ingestion of MPs remain unclear. Neutrophils express various families of adhesion molecules, such as integrins and selectins, that interact with their corresponding ligands on other cells or in the extracellular matrix, leading to the formation of transient or stable adhesions (37–39). In addition, the adhesion of neutrophil could be induced by the polarization stimulated by the pathogens and local signals of tissue damage. Thus, it is hypothesized that MPs as xenobiotics cause neutrophils polarization, facilitating their interaction with endothelial cells and resulting in adhesion (40, 41). In addition, the formation of neutrophil extracellular traps may occur subsequent to phagocytosis and adhesion (42). As we observed the cellular morphology characterized by umbrella-like folds in the 3D imaging (Fig. 5D). Moreover, it could also explain why the obstructions were observed in such a fast time; in addition to the role of the vascular environment, changes in the adhesive properties of the MPL-Cells themselves may be key. The ingestion of the larger particles may induce changes in cell size and physical attributes, potentially leading to swelling or increased hardness (43, 44), making it more challenging for them to pass through the vascular stenosis and the turns. Consistent with this, the flow cytometry data showed that the neutrophils with MPs revealed an increase in FSC and a decrease in SSC (Fig. 4, G and H).

However, multiple cellular biological processes are involved in the MPL-Cell obstruction, most of which remain unknown. It is unclear which cellular signaling pathways are altered following the phagocytosis of MPs and which cell surface receptors are subsequently affected. Furthermore, the role of these membrane receptors in the process of cell obstruction is not well understood. Whether the specificity of the vascular endothelium regulates the obstruction. The extent to which signaling proteins in blood influence this process remains to be determined. However, the inhibition of specific

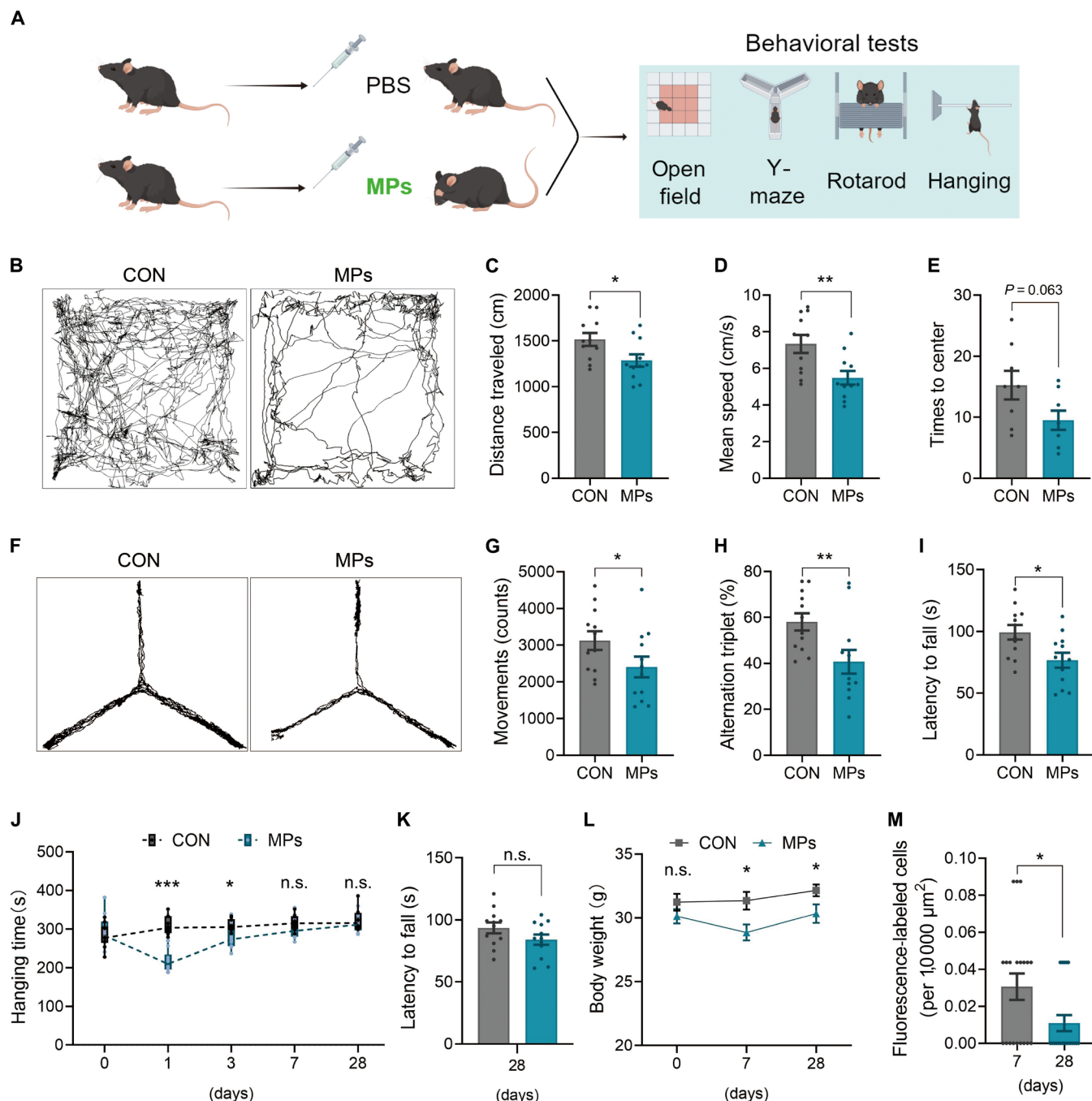


Fig. 8. Suppressed blood flow in the brain causes neurobehavioral disorders. (A) Schematic representation of the arrangements for behavioral experiments. Created using FigDraw.com. (B to E) An open-field experiment was performed on MP-treated and control mice ($n = 10$ to 11 mice for each group), representative diagrams of mouse locomotor trajectories (B), analytical statistics of the total travel distance (C), movement speeds (D), and the number of trips to the center zone (E). (F to H) Y-maze experiment ($n = 11$ mice for each group), representative diagrams of mouse locomotor trajectories (F), analytical statistics of the total movements (G), and alternation triplet (H). (I) The latency time in the rotarod test of the MP-treated and control mice ($n = 12$ mice for each group). (J) Hanging time for MP-treated mice and control mice experimented on different days after MP treatment ($n = 12$ mice for each group). (K) Latency time in the rotarod test at 28 days after MP treatment ($n = 12$ mice for each group). (L) Body weight change curves of mice affected by MP treatment ($n = 11$ to 12 mice for each group). (M) Analytical statistics of the obstructed MPL-Cell density in the brain at 7 and 28 days after MP treatment ($n = 20$ FOVs from four mice per time point). Data are represented as means $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

functional proteins aids in elucidating the cellular biological processes that may be involved in this phenomenon. For instance, inhibiting the functional protein dynamin with the small-molecule drug Dyngo-4a may provide insights into how endocytosis affects MPL-Cell levels. In addition, inhibiting cell adhesion using the small-molecule drug Milategrast could be used to investigate whether the obstruction of MPL-Cells is dependent on the cell adhesion or whether the structural expansion of the cells alone is sufficient to induce the obstruction phenomena at this scale. Additional further molecular manipulations should be used to explore the underlying mechanisms. We have existing data indicating that the size of phagocytosed particles is critical for triggering cellular obstruction (Fig. 6B); however, it remains unclear whether because particles of varying sizes activate different signaling pathways, thereby influencing cell attachment. In addition, *in vitro* experiments have demonstrated that the size of phagocytic particles directly affects immune cell functionality (43). Extraction and transcriptome sequencing analysis of PL-Cells induced by plastic particles of differing sizes will help further elucidate this issue.

The obstruction of the MPL-Cells is characterized by dynamic changes. In certain instances, MPL-Cells could not remain stable after obstruction and exhibit slow movement within the vessel. Following a period of interaction with the vessel wall, they may detach and continue to move with the blood flow (Fig. 3I). On the other hand, an MPL-Cell that has become stably obstructed can impede the movement of newly arriving MPL-Cells, causing them to coalesce and increase the area of the obstruction (movie S4). However, most of the newly arriving MPL-Cells disengage and continue moving after a certain duration. Notably, an MPL-Cell that has become stably obstructed will not be affected by the presence of a new MPL-Cell nor will it be displaced. This stability likely arises from a relatively stronger bond formed between the MPL-Cell and the vessel wall. Similarly, a developing thrombus recruits unstimulated platelets. Thrombus formation is a dynamic process in which some platelets adhere to the developing thrombus, while others separate from it, influenced by shear, flow, and turbulence (45, 46). However, the progression from MPL-Cell to thrombus may represent a distinct pathway compared to the classical thrombus formation triggered by vascular injury, which initiates platelet activation through subendothelial collagen (47). The changes induced by MP in the MPL-Cell itself, leading to obstruction, should be the primary causative event and first to occur. Afterward, the immune factors released by MPL-Cells may collaborate with tissue factors, triggering a secondary pathway that initiates platelet activation and, subsequently, activates a proteolytic cascade that generates thrombin. Visualizing platelets during the obstruction of MPL-Cells through fluorescent labeling will help address this question.

The obstruction of MPL-Cells rapidly decreased blood perfusion levels in the brain. We did not observe longer-term changes, and the data at 50 min already showed no significant differences from the data at 30 min (Fig. 7B). In addition, subgroup analyses of different flow vessels suggest that the blockage effect is more pronounced in narrower vessels with lower perfusion rates (Fig. 7D), supporting the idea that the inhibition of blood perfusion is caused by cellular obstruction. The specific structure of the vascular network in the cortex means that obstruction of key nodes can directly affect perfusion levels in a specific area of tributaries (48, 49). This explains the results that the bottleneck period of inhibition occurs rapidly. The blocked cells, which were significantly reduced after 4 weeks, were

not completely eliminated. Correspondingly, behavioral deficits in mice returned to baseline levels after 4 weeks.

It was believed that MPs influence brain function in two primary ways. MPs can indirectly regulate brain function through peripheral organs, or they might influence the brain by somehow crossing the BBB or interacting directly with it. The MPL-Cell mechanism can be categorized as a third type of potential regulation in which MPs affects brain function; unlike the previously thought, MPs reach local brain tissues but do not cross the BBB and affect local brain function by regulating blood perfusion. Our study has identified an ingenious mechanism through which MPs make function to organisms. We have demonstrated a negative effect of this mechanism on neurobehavioral regulation in the brain; moreover, we also believe that it may also have detrimental impacts on a broader range of organs. Environment MPs might be another important risk factor for cardiovascular disease (18, 50, 51) and received scant attention so far. Our findings provide a mechanistic explanation for tissue damage induced by MPs, particularly regarding their effects on vascular obstruction. This obstruction is likely to have detrimental consequences for cardiovascular health and may result in more severe adverse effects, especially in patients with underlying conditions similar to myocardial infarction. Notably, a recent study on humans by Marfella *et al.* (18) supports this correlation.

However, it is premature to directly apply this mechanism to human research systems. Humans and mice have different immune systems, coagulation systems, and cardiovascular and cerebrovascular circulatory systems (52–55). The circulating blood volume in humans is ~1200 times greater than that of mice, and, notably, significantly different vascular diameters would greatly reduce the degree of MPL-Cell obstruction in humans. The internal diameter of the coronary arteries in the human heart is about 4 mm, whereas, in mice, it measures less than 100 μm (56, 57). Consequently, there is uncertainty regarding whether MPs will induce or influence the obstruction in human blood vessels. However, the diameter of human capillary microvessels at their narrowest point measures only 8 to 10 μm , which closely resembles the 8- to 9- μm diameter of the terminal branches of venous vessels in mice (58–60). Moreover, we can deduce that the presence of MPs in the bloodstream may more likely induce obstruction in areas where there are sedimentations in the lining of blood vessels. These preexisting conditions could hinder the transit of MPL-Cells through narrowed vascular regions, thereby increasing the likelihood of obstruction. This may significantly elevate the risk for specific populations, particularly those with a history of thrombotic conditions, such as cerebrovascular infarction and myocardial infarction. In these patients, MPL-Cells are more likely to cause obstructions at the narrowed sites of blood vessels. Obesity is increasingly as a prevalent human disease, and it can contribute to the accumulation of cholesterol and lipids in the walls of blood vessels (61, 62). Therefore, it is essential to investigate the potential obstructive effects of MPL-Cells in humans. Moreover, humans are exposed to a variety of MP sizes, and the combinatorial effects of different MP sizes on the likelihood of inducing infarctions remain to be estimated. The use of larger mammals or animal models that more closely resemble the human circulatory system, such as nonhuman primates, is thus crucial for studying this process.

In this study, we investigated the effects of MPs exposure over a maximum duration of 28 days. The long-term consequences of MPL-Cell obstruction and the cumulative effects of multiple repeated MPs exposures remain unknown. Collectively, studies indicate that the

human circulatory system is at significant risk due to direct exposure to MPs (63, 64). If medical injection devices are not rapidly and thoroughly improved, then the direct infiltration of MPs into the human bloodstream may become a persistent and potentially recurrent issue. The potential long-term effects of MPs on neurological disorders such as depression and cardiovascular health are concerning. This study offers a theoretical foundation and a focused direction for understanding the potential health risks associated with MPs in this context. Increased investment in this area of research is urgent and essential to fully comprehend the health risks posed by MPs in human blood.

METHODS

Mouse husbandry and experimentation

All experiments involving animals conformed to the rules of the Association for Assessment and Accreditation of Laboratory Animal Care and the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication eighth edition, update 2011). All procedures were approved by the Animal Care Committee of Charles River (PA23053002-0) and PKU-Nanjing Institute of Translational Medicine (IACUC-2021-023). All mice were housed in a temperature-controlled (20° to 22°C) and specific pathogen-free animal facility, three to five per cage, and maintained on a 12-hour light/dark cycle, with water and food available ad libitum.

Eight-week-old male wild-type (WT) C57BL/6J mice were procured from Vital River Laboratory. We used a 5- μ m-diameter fluorescently labeled polystyrene plastic microsphere, MPs, a commonly used material in the field (65–69) (Baseline, catalog no. 7-3-0500). On the basis of previous research, mice were assigned randomly to untreated or gavaged with 100 μ l of MP water mixture at a dose of 2 mg/ml. Experimenters were informed about the grouping of mice. MPs can enter the human bloodstream through medical supplies (8, 70–72), about 12 μ g of MPs per milliliter of blood have been detected in human blood. The need to simulate the human condition in the concentration settings of the experiment was taken into account. We would like to bring mouse blood MPs to this level by injection. An adult mouse of 30 g has a blood volume of about 2 ml. We injected 100 μ l of MPs at a concentration of 1 mg/ml intravenously into the mouse, and the diluted final concentration after entering the bloodstream should be blood of about 50 μ g/ml. In the experiments involving varying concentrations of MPs exposure, the injection volume remained constant, while the MP concentrations administered were diluted to 0.5 and 0.1 mg/ml. In the exposure experiments with different sizes of plastic particles, 2- μ m-diameter (Baseline, catalog no. 7-3-0200) and 80-nm-diameter (Baseline, catalog no. 7-3-0008) fluorescently labeled polystyrene plastics were used.

Male WT mice, 8 weeks old, were procured from Vital River Laboratory. After a 2-week acclimatization period to the rearing environment, the mice were used for behavioral detection experiments. To minimize the impact of injection manipulation on mice, WT mice designated for behavioral assays were not permitted to undergo behavioral testing until at least 6 hours after the completion of intravenous injection. Generally, all injections in both groups of mice were administered within a controlled timeframe of 1 hour. Control mice received an equivalent volume of phosphate-buffered saline (PBS) solution.

Surgery

All surgical procedures were performed under sterile conditions, and all reagents administered to the animals were sterile. Mice were

anesthetized with 1.5% isoflurane in air at a flow rate of 0.4 liters/min and kept on a 37°C heating pad during surgery. The specific cerebral cortical region for imaging was identified using stereotactic coordinates (motor cortex area, from bregma: anteroposterior, 0.5 mm; mediolateral, 1.2 mm). The skull over the ROI was thinned using a high-speed micro-drill under a dissection microscope, with intermittent drilling and application of normal saline to prevent overheating and damage to the underlying cerebral tissue. After removing the external layer of compact bone and most of the spongy bone layer, the area was further thinned until a smooth, expanded area was achieved, ensuring that the center was thin enough for high-quality imaging. A drop of saline was applied, and a 3-mm-diameter glass coverslip, sterilized in 70% ethanol, was placed on the window. Dental cement was then applied around the glass coverslip. All subjects were housed individually in separate cages following the surgery. The mice were used in the experiment after a postoperative recovery period of 2 to 4 weeks.

AAV9-hSyn-DIO-mitoEGFP (100 nl; BrainVTA, no. PT-2619) and AAV9-CaMKII-Cre (100 nl; BrainVTA, no. PT-0220) were injected into the motor cortex at coordinates—anteroposterior, 0.5 mm; mediolateral, 1.2 mm; dorsoventral, –0.3 mm—at a rate of 50 nl/min. A 3-mm-diameter glass coverslip was implanted and secured on the motor cortex. The mice were used in the experiment 2 to 4 weeks after postoperative recovery.

Imaging and recording

After undergoing surgery, mice fitted with a transparent cranial window were selected for further experiments. The mice were securely head fixed on the imaging stage, and single-photon imaging was initially conducted to determine the appropriate vascular FOV. Subsequently, mTPM was used. A suitable FOV containing a clear image of blood vessels located 20 to 50 μ m beneath the pial surface was identified. The mTPM and FOV were then stabilized, and changes following the administration of MP treatment were observed within the same imaging FOV. The fast high-resolution mTPM large FOV model featured a headpiece weight of 2.45 g, a lens numerical aperture of 0.5, and an FOV of 520 μ m by 440 μ m. Images were captured to visualize cortical blood vessels surveillance through either a time-lapse xy imaging stack at a frame rate of 4.8 frames per second or multiplane imaging stacks consisting of 40 planes at 2.5- μ m intervals, with 4.8 frames per second. Each mTPM model was equipped with a water-immersion miniature objective and a 920-nm femtosecond fiber laser. Fluorescence imaging excitation was achieved with 150-fs laser pulses (80 MHz) at 920 nm for the fluorescently labeled MPs, with a power of ~25 mW after passing through the objective.

Blood sample processing and white blood cell isolation

Upon receipt, ~700 μ l of fresh blood samples were received in EDTA tubes, within 2 hours of collection at room temperature (RT) mixed with 6.3 ml of 10 $\times$ red blood cell (RBC) lysis buffer (Beyotime, catalog no. C3702). RBC lysis of whole blood was performed to isolate leukocytes. Samples were lysed at RT for 10 min, continuously mixing on a tube rotator. Cells were then centrifuged at 300g for 5 min and washed with Sorter Buffer (PBS and 1 mM EDTA). The cells were washed twice with PBS and then used for the next step.

Flow cytometry

Cells were stained with specific surface or intracellular antibodies (shown in the following) in staining buffer (1.0% bovine serum

albumin). Cells were washed three times by PBS. The antibodies used were anti-CD45 (BioLegend, 47-0451-80), anti-F4/80 (Life Technologies, 11-4801-81), and anti-Ly6G/Ly6C (BD Biosciences, 561103). Cells were analyzed on an LSRFortessa (BD) flow cytometer, and data were analyzed using FlowJo X software.

Laser speckle contrast imaging

Mice were imaged under isoflurane anesthesia (3% induction, 1 to 1.5% maintenance, in oxygen) by head fixed, and the skull was exposed. Camera focus and exposure time were adjusted to achieve optimal imaging conditions. After locking the imaging FOV, mice were injected intravenously with MPs. Tests were performed at different time points after injection. For each imaging, time-length 1-min blood perfusion levels were continuously recorded. The mean perfusion level within 1 min for each ROI was calculated.

Open field

The dimensions of the open field for the mice are 50 cm in length, 50 cm in width, and 40 cm in height. The laboratory environment is maintained in a quiet state with controlled lighting at about 8 lux. Before the experiment, the mice were acclimatized to the laboratory environment for at least 30 min to minimize stress response to the unfamiliar surroundings. At the start of the experiment, the mice were gently placed in a corner of the open field, with each mouse starting from the same initial position. The behavioral activities of the mice were recorded via filming for a duration of 7 min. The sequence in which the mice from the two experimental groups underwent the experiment was randomized. After the completion of the experiment for each mouse, any residual urine in the experimental area was cleaned using alcohol. Following the cleanup, the alcohol odor was allowed to dissipate before proceeding with the next mouse.

Y-maze

The Y-maze consisted of a Y-shaped compartment (21 cm by 7 cm by 15.5 cm), it is three arms of equal length. The mice were acclimatized to the laboratory environment for 3 days before the experiment to familiarize themselves with the room and reduce stress. The arms of the maze were labeled A, B, and C. Mice were gently placed in the fixed arm facing the center of the maze. They were given 8 min to explore the maze freely. The maze was thoroughly cleaned with alcohol between experimental mice.

Rotarod test

The rotarod test was used to evaluate the mice's ability to maintain balance on a rotating rod. As the rotational speed increased, it became more challenging for the mice to stay balanced. Before the test, the mice underwent a 2-day training period, with three sessions per day, where they were trained to walk on a rotating rod at a speed of 5 rpm/min for 3 min each time. During the actual test, the mice were placed on the rotarod, and the rotational speed was gradually increased from 5 rpm/min to 30 rpm/min over a period of 4 min. The latency time, indicating when the mice fell due to their inability to maintain balance, was recorded during the acceleration.

Rod-hanging test

Mice were allowed to grasp a horizontally maintained 2-mm-diameter wooden rod with their four paws, positioned about 50 cm above a thick layer of soft bedding. The time until the mice fell from the rod was recorded. Following each fall, the mice were permitted to recover for

2 min. Each mouse session comprised three trials, and the hanging time was averaged. To ensure consistency in testing, experiments typically commenced at 19:00 and concluded by 22:00 on each experimental day.

Quantification and statistical analysis

Imaging data were processed with Fiji (v1.54). All data are shown as the means $\pm$ SEM. All boxes showed the median of the data. n denotes the number of biological replicates in each experiment, and it is provided in the corresponding figure legends. All statistical analyses were conducted using GraphPad Prism (v8.0.2). All two-group comparisons were calculated using Student's t test (two-tailed, paired, or unpaired). The results were considered statistically significant when $P < 0.05$.

Supplementary Materials

The PDF file includes:

Figs. S1 to S7

Legends for movies S1 to S7

Other Supplementary Material for this manuscript includes the following:

Movies S1 to S7

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