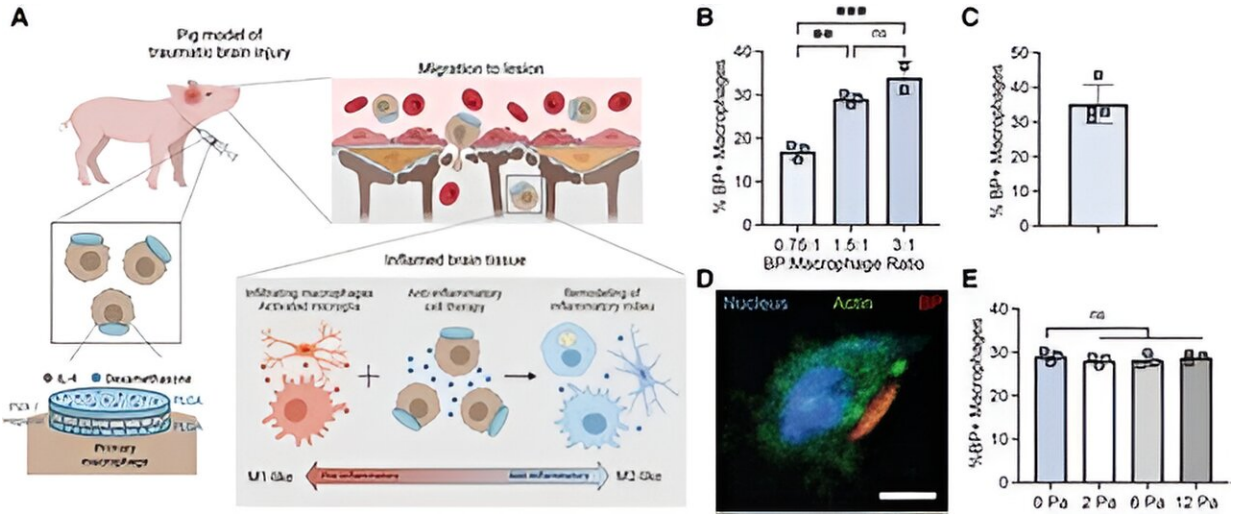
Using the body's own cells to treat traumatic brain injury

January 9 2024



Backpacks durably adhere to porcine macrophages. A) The schematic of the concept shows that backpacks loaded with IL-4 and dexamethasone are adhered to porcine macrophages. Upon intravenous infusion, backpack–macrophages respond to inflammatory chemotactic cues to migrate to and traverse across the disrupted blood–brain barrier. Backpack–macrophages extravasate into the inflamed brain lesion to remodel the inflammatory milieu. Credit: *PNAS Nexus* (2023). DOI: 10.1093/pnasnexus/pgad434

Scientists have created a new treatment for traumatic brain injury (TBI) that shrank brain lesions by 56% and significantly reduced local inflammation levels in pigs. The new approach leverages macrophages, a type of white blood cell that can dial inflammation up or down in the

body in response to infection and injury.

The team created disk-shaped microparticles called "backpacks" containing anti-inflammatory molecules, then attached them directly to the macrophages. These molecules kept the cells in an anti-inflammatory state when they arrived at the injury site in the brain, enabling them to reduce local inflammation and mitigate the damage caused.

The research is reported in *PNAS Nexus*.

"Every year, millions of people suffer from a TBI, but there is currently no treatment beyond managing symptoms. We have applied our cellular backpack technology—which we previously used to improve macrophages' inflammatory response to cancerous tumors—to deliver localized anti-inflammatory treatment in the brain, which helps mitigate the cascade of runaway inflammation that causes tissue damage and death in a human-relevant model," said senior author Samir Mitragotri, Ph.D., in whose lab the research was performed.

Mitragotri is a Core Faculty member of the Wyss Institute at Harvard University and the Hiller Professor of Bioengineering and Hansjörg Wyss Professor of Biologically Inspired Engineering at Harvard's John A. Paulson School of Engineering and Applied Sciences (SEAS).

Stopping a runaway inflammation train

More than a million people in the US suffer from a traumatic brain injury (TBI) every year, about 230,000 of them are hospitalized, and almost 70,000 die from TBI-related causes.

There is currently no treatment for the damage caused to brain tissue during a TBI, beyond managing a patient's symptoms. One of the main drivers of TBI-caused damage is a runaway inflammatory cascade in the

brain.

As cells die from the impact, they release a cocktail of pro-inflammatory cytokine molecules that attract immune cells to clean up the damage. But the same cytokine molecules can also disrupt the blood-brain barrier, which causes blood to leak into the brain. Blood accumulation in the brain causes swelling, impaired oxygen delivery, and increased inflammation, and creates a vicious cycle of bleeding and damage that drives even more cell death.

The Mitragotri lab saw an opportunity in this problem.

"It's generally believed anti-inflammatory therapies can be effective for treating TBI, but so far, none of them have proven effective clinically. Our previous work with macrophages has shown us that we can use our backpack technology to effectively steer their behavior when they arrive at the injury site," said co-first author Rick Liao, Ph.D., a Postdoctoral Fellow at the Wyss Institute and SEAS.

"Since these cells are already active players in the body's natural immune response to a TBI, we had a hunch we could augment that preexisting biology to reduce the initial damage."

Macrophages are very malleable cells and can "switch" between pro-inflammatory and anti-inflammatory states. While the team's previous work in cancer had been focused on keeping macrophages in a pro-inflammatory state when they arrive at the inflammation-reducing microenvironment of a tumor, this new project would be trying to do the opposite: keep the macrophages "calm" in the inflammation-riddled setting of a brain injury.

To do so, they used a disk-shaped "backpack" they had previously designed to treat multiple sclerosis that contained layers of two anti-

inflammatory molecules: dexamethasone, a steroid, and interleukin-4, a cytokine that encourages macrophages to adopt an anti-inflammatory state.

They then incubated these microparticles with both human and pig macrophages in vitro and saw that the backpacks stably stuck to the cells without causing any negative effect. They also observed that application of their backpacks decreased the expression of pro-inflammatory biomarkers and increased the expression of anti-inflammatory biomarkers, retaining the pig macrophages in a healing state.

But to prove that this shift would work in the body, they had to test the backpack-bearing macrophages in vivo. They chose pigs as their model organism because their brains' structures and responses to injury more closely mimic those of humans than mice.

"Probably our biggest challenge in this project was scaling up production to match what we needed to run the experiments. Our previous studies were done in rodents, which required about two million macrophages and four million backpacks administered per subject," said co-first author Neha Kapate, Ph.D., a Postdoctoral Fellow at the Wyss Institute and SEAS.

"For the porcine study, we needed 100 million macrophages and 200 million backpacks per subject—on the scale of what would be administered in humans—and lots of helping hands." The final team consisted of over 20 members from across the Wyss Institute, Harvard, MIT, and Mass General Hospital (MGH).

Once they had generated enough backpack-wearing porcine macrophages, they infused them into the pigs' bloodstreams four hours after a TBI. Seven days later, they analyzed the animals' brains.

Pigs that had received the macrophage treatment showed a high concentration of the cells in the area immediately surrounding the injury site, their lesions were 56% smaller, and there was significantly less hemorrhaging than in untreated animals.

Local immune cells also displayed a lower amount of a pro-inflammatory activation marker called CD80, indicating that the macrophages had accomplished their damage control by reducing inflammation in the brain. Corroborating that data, the levels of two soluble biomarkers for inflammation in the blood and cerebrospinal fluid were lower in treated animals than in untreated animals. The macrophage treatment also did not cause any negative effects.

The team plans to conduct future studies that focus on elucidating exactly how their anti-inflammatory macrophage therapy affects the blood-brain barrier's integrity to prevent bleeding, which could also hold promise for treating other conditions like hemorrhagic strokes.

"Macrophages' susceptibility to their local environment has historically prevented scientists from taking full advantage of their immune-modulating capabilities," said Wyss Founding Director Donald Ingber, M.D., Ph.D.

"This impressive study describes a truly novel and potentially powerful macrophage-based therapy for treating the inflammation that is the root cause of so many human afflictions in an effective and non-invasive way that works with biology rather than against it." Ingber is also the Judah Folkman Professor of Vascular Biology at Harvard Medical School and Boston Children's Hospital, and the Hansjörg Wyss Professor of Bioinspired Engineering at SEAS.

More information: Neha Kapate et al, Backpack-mediated anti-inflammatory macrophage cell therapy for the treatment of traumatic

brain injury, *PNAS Nexus* (2023). DOI: 10.1093/pnasnexus/pgad434