Implantation of Neural Stem Cells Carrying Galectin-1 Elicits Long-term Neuroprotection Against Traumatic Brain Injury in Mice

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Introduction

Although traumatic brain injury (TBI) is a major public health problem with a deep socioeconomic impact, no effective therapies have been developed to date. The extracellular matrix (ECM) is a highly dynamic non-cellular structure present in all tissues and organs. The ECM provides three-dimensional physical support, segregates cells and tissues, and transmits biological signals for cell proliferation, adhesion, and migration. ECM supports the survival of stem cells and has been successfully used as a delivery vehicle in stem cell transplantation. However, it is not known if brain ECM facilitates tissue repair and functional recovery after TBI in animal models.

Methods

Male C57BL/6J mice (8-10 weeks old) were purchased from the Jackson Laboratory. ECM derived from porcine brains was prepared as described previously. TBI was induced by a controlled cortical impact (CCI). The CCI was centered 2.0 mm lateral to midline and 2.0 mm anterior to bregma, and was produced with a pneumatically driven CCI device (Precision Systems and Instrumentation, Fairfax, VA, USA) using a 3-mm flat-tip impounder (velocity, 3.75 m/sec; duration, 150 msec; depth, 1.5 mm). One hour after surgery, each mouse received two injections, into the right corpus callosum (AP: 1.10 mm; ML: 1.0 mm; DV: 1.5 mm) and the striatum (AP: -0.80 mm; ML: 1.5 mm; DV: 3.5 mm). To examine the distribution of the ECM after injection, 1 μL of a mixture of Evans blue (0.5 mg/mL in water) and ECM (5 μg/mL in PBS) at a volume of 1:1 was injected into the brain at 1 h after CCI as described above. Thick coronal sections (1 mm) were cut to view the anatomical distribution of the Evans blue dye. Behavior test including Rotarod test, Wire hang test, Corner test, Morris water maze test were performed postsurgery. At 35 d after TBI, mice were deeply anesthetized and transcardially perfused with 0.9% NaCl followed by 4% paraformaldehyde in PBS. Brains were cryoprotected in 30% sucrose in PBS, and frozen serial coronal brain sections (25 μm) were cut on a cryostat. Immunohistochemistry was performed on free-floating sections.

Figure 1. ECM treatment reduces TBI lesion volume.

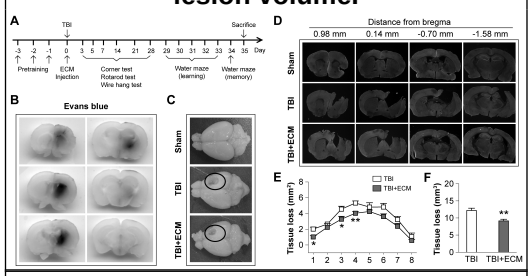


Figure 2. ECM treatment confers long-term protection against TBI-induced neurobehavioral deficits.

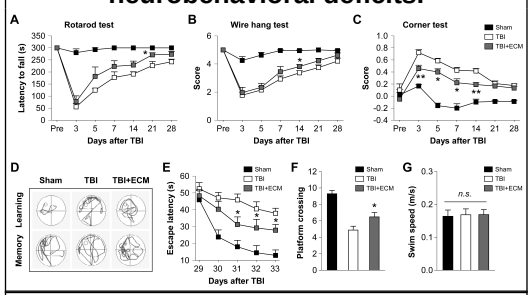


Figure 3. ECM treatment reduces hippocampal CA3 neurodegeneration after TBI.

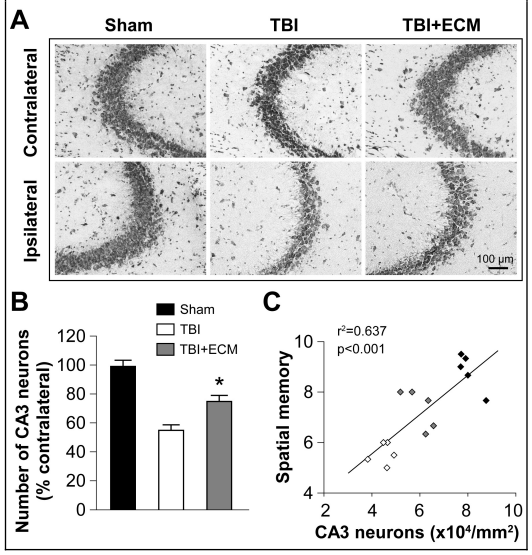
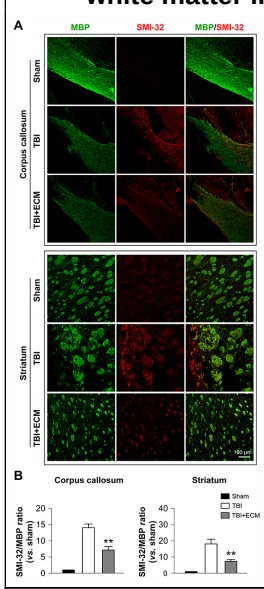


Figure 4. ECM implantation ameliorates white matter injury after TBI.



Mice were subjected to TBI and received ECM implantation 1 h after TBI. Brain sections were dual-stained for MBP (green) and non-phosphorylated neurofilament H (SMI-32; red) 35 d after TBI. (A) Representative immunofluorescent images of MBP and SMI-32 staining in the corpus callosum and striatum. (B) The degree of white matter injury was quantified by the ratio of SMI-32 to MBP and expressed relative to sham controls. n=4 mice/group. **p≤0.01 TBI+ECM versus TBI.

Figure 5. ECM implantation reduces reactive astrogliosis and glial scar formation after TBI.

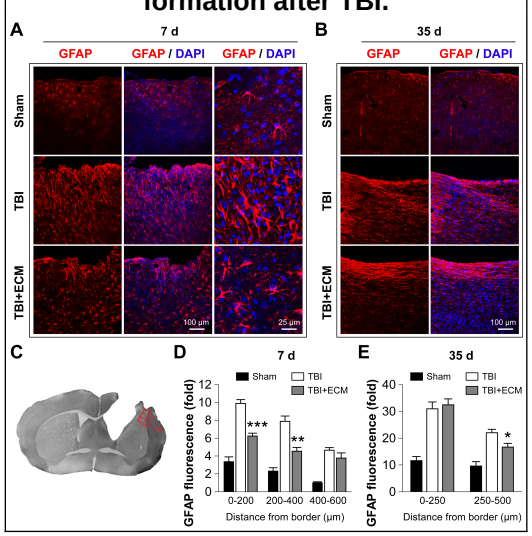
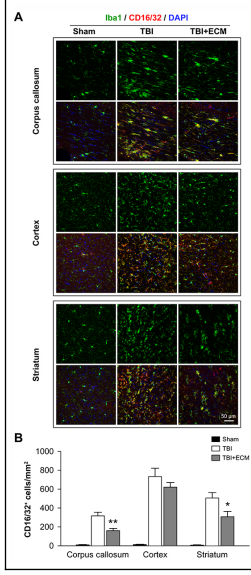


Figure 6. ECM implantation suppresses pro-inflammatory microglial/macrophage activation after TBI.



Mice were subjected to TBI and received ECM implantation 1 h after TBI. Brain sections were dual-stained for microglial/macrophage marker Iba1 and M1 microglial/macrophage marker CD16/32 at 10 d after TBI. (A) Representative immunofluorescence images of CD16/32 (red) and Iba1 (green) staining in the corpus callosum, cortex, and striatum. Sections were counterstained with DAPI (blue) for nuclear labeling. (B) Quantification of CD16/32+ cells. n=5 mice/group. *p≤0.05, **p≤0.01 TBI+ECM versus TBI.

Conclusion

ECM ameliorated TBI-induced neurodegeneration and white matter injury. Furthermore, ECM treatment mitigated glial scar formation and proinflammatory microglial responses, thereby promoting tissue remodeling and repair. In summary, brain ECM may be an ideal scaffold material for local transplantation and injection in future studies to achieve a more complete, full-scale recovery after TBI.

Acknowledgements

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