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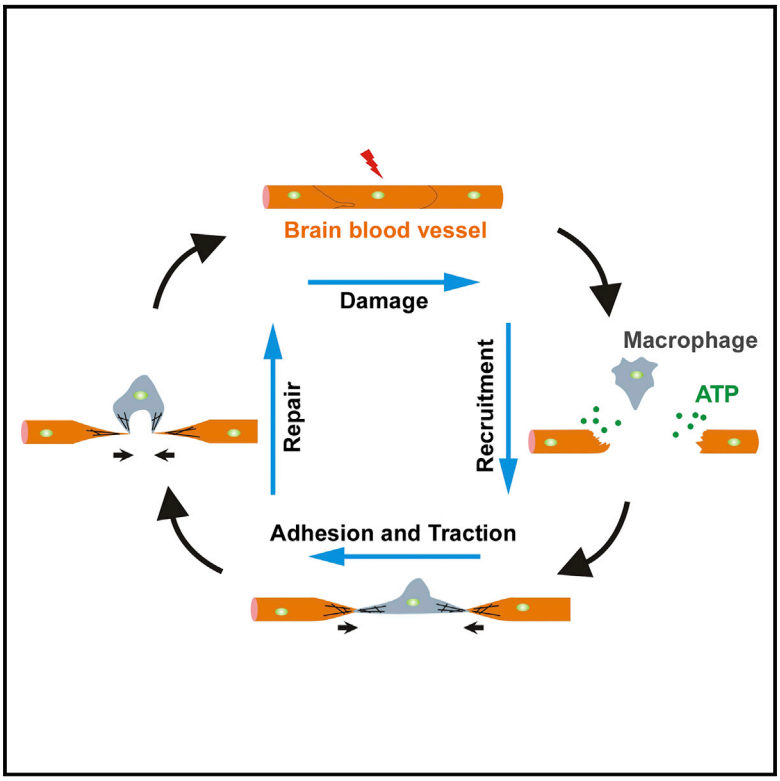
# Macrophages Mediate the Repair of Brain Vascular Rupture through Direct Physical Adhesion and Mechanical Traction

## Graphical Abstract

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## In Brief

Luo and colleagues reveal a hitherto unexpected role of macrophages in the repair of brain vascular ruptures. Once rupture of brain blood vessel occurs, macrophages come to mediate the repair of the rupture through direct physical adhesion to the breaking points and generation of mechanical traction forces.

## Highlights

- Zebrafish brain vascular rupture and repair system is established
- Live imaging reveals the dynamic cellular events of brain vascular repair
- Macrophages mediate brain vascular repair through adhesion and mechanical traction
- Macrophage-mediated brain vascular repair requires PI3K and Rac1 activity

## Accession Numbers

GSE78194

Liu et al., 2016, Immunity *44*, 1162–1176
May 17, 2016 
http://dx.doi.org/10.1016/j.immuni.2016.03.008

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# Macrophages Mediate the Repair of Brain Vascular Rupture through Direct Physical Adhesion and Mechanical Traction

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http://dx.doi.org/10.1016/j.immuni.2016.03.008

## SUMMARY

**Hemorrhagic stroke and brain microbleeds are caused by cerebrovascular ruptures. Fast repair of such ruptures is the most promising therapeutic approach. Due to a lack of high-resolution in vivo real-time studies, the dynamic cellular events involved in cerebrovascular repair remain unknown. Here, we have developed a cerebrovascular rupture system in zebrafish by using multi-photon laser, which generates a lesion with two endothelial ends. In vivo time-lapse imaging showed that a macrophage arrived at the lesion and extended filopodia or lamellipodia to physically adhere to both endothelial ends. This macrophage generated mechanical traction forces to pull the endothelial ends and facilitate their ligation, thus mediating the repair of the rupture. Both depolymerization of microfilaments and inhibition of phosphatidylinositide 3-kinase or Rac1 activity disrupted macrophage-endothelial adhesion and impaired cerebrovascular repair. Our study reveals a hitherto unexpected role for macrophages in mediating repair of cerebrovascular ruptures through direct physical adhesion and mechanical traction.**

## INTRODUCTION

Pathological bleeding in the brain includes hemorrhagic stroke and microbleeds. Hemorrhagic stroke, one of the leading causes of death and adult disability worldwide, is primarily caused by hypertensive atherosclerosis followed by the rupture of cerebral arterioles. Microbleeds result from ruptures in microvessels in the brain. Recently, improvements in imaging techniques have enabled the detection of previously invisible microbleeds. Although brain microbleeds do not present with acute symptoms as in major strokes, they are closely associated with age-related cognitive decline and dementia (Poels et al., 2012; Gregoire et al., 2012; Liman and Endres, 2012; Nishimura and Schaffer, 2013). Once these cerebrovascular ruptures have occurred, fast repair of the ruptures is clinically necessary to avoid the exacerbation of symptoms and becomes the most efficacious therapeutic approach for treating these ruptures.

Damage to endothelial integrity is the key reason for cerebrovascular ruptures (Deanfield et al., 2007). Thus, the maintenance of brain vascular health requires the fast repair of endothelial lesions and the recovery of endothelial integrity. Endothelial repair can be achieved through the self-replication of endothelial cells (ECs) and the differentiation of bone marrow-derived endothelial progenitor cells (Asahara et al., 1997). However, these two mechanisms normally take place during ischemic vascular regeneration and always involve therapeutic neoangiogenesis. The cellular mechanisms underlying the repair of cerebrovascular ruptures remain to be elucidated.

Macrophages are associated with brain injury, hemorrhage, and several aspects of vascular development and remodeling. After brain injury, ATP released from the injured parenchymal tissues mediates a rapid response of microglia, the principal immune cells in the brain (Davalos et al., 2005; Sieger et al., 2012). Macrophages play central roles in inflammation and phagocytosis through their ability to recognize and engulf apoptotic cells. After spinal cord or brain injury, macrophages are essential for regenerative response and recovery (Shechter et al., 2009, 2013; Kyritsis et al., 2012). In murine stroke models, macrophages prevent hemorrhagic infarct transformation (Gliem et al., 2012). Patients with stroke have high amounts of macrophages and inflammatory cytokines in the plasma around stroke lesions (Sánchez-Moreno et al., 2004; Chiba and Umegaki, 2013). Most capillary microbleeds in the elderly also involve macrophages (Fazekas et al., 1999; Fisher et al., 2010). During angiogenesis, macrophages are thought to promote the fusion of endothelial tip cells. However, because this fusion is unaffected in macrophage-deficient mice, macrophages might promote fusion via assistance or stabilization rather than a direct "bridge" (Checchin et al., 2006; Kubota et al., 2009; Fantin et al., 2010; Schmidt and Carmeliet, 2010; Rymo et al., 2011). This conclusion has been verified by the finding that macrophages secrete vascular endothelial growth factor (VEGF)-C to stabilize tip cell fusion and increase vascular complexity (Tammela et al., 2011; Geudens and Gerhardt, 2011). Until now, there has been no direct evidence that macrophages are absolutely required for angiogenesis.

Direct interactions between macrophages and ECs play important roles in inflammation and atherosclerosis. To reach the site of inflammation, macrophages in the circulation need