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New and Notable

Nervous Tissue Stiffens Postinjury

Ellen Kuhl[1,*]
[1]Departments of Mechanical Engineering and Bioengineering, Wu Tsai Neurosciences Institute, Stanford University, Stanford, California

Have you ever seen a zebrafish in a wheelchair? Most likely not. Unlike mammals, zebrafish have an almost limitless regenerative potential and can faithfully regrow amputated fins; regenerate their pancreas, liver, and kidney; repair their heart; and—after traumatic spinal cord injury—fully recover from temporary paralysis (1). Spinal cord repair in zebrafish relies on two mechanisms: axonal regeneration, the regrowth of axonal projections from existing neurons; and neuronal regeneration, the creation of new neurons from neuronal stem cells (2). We now know that glial cells play a central role in this process. They proliferate and infiltrate the lesion, divide and migrate into the damaged site, and connect the two sides of the injury to guide new axons, a mechanism that is known as glial bridge (3). To no surprise, throughout the past decade, zebrafish have become a primary model system to study spinal cord injury and repair. Can zebrafish teach us how to promote adult neurogenesis and stimulate successful spinal cord repair in humans?

In humans, traumatic spinal cord injury causes an irreversible loss of neurons and permanent paralysis. There is currently no treatment to repair the damaged tissue and restore lost function (4). Approximately 500,000 people suffer from spinal cord injury annually, and the number of people living with this condition exceeds 250,000 in the United States alone (3). In traumatic spinal cord injury, a primary physical insult damages the neuronal cells and initiates a complex secondary injury cascade associated with progressive cell death, ischemia, and inflammation. Over time, the injury site will undergo microstructural changes, including the formation of cystic cavities and glial scars (4). For more than a decade, glial scarring was believed to create an inhibitory microenvironment that prevents neurite outgrowth, cell migration, and axonal regeneration (5). Although there is a general agreement that a supportive microenvironment is critical to successfully repair the spinal cord, the molecular mechanisms that trigger spinal cord regeneration in zebrafish and inhibit spinal cord repair in humans remain poorly understood (6).

In this issue of *Biophysical Journal*, Möllmert et al. (7) present the first systematic characterization of the biochemomechanical microenvironment of the spinal cord in response to spinal cord transection. Using a chronic zebrafish model, the authors thoroughly characterize regional variations in gray and white matter stiffness, transient stiffness changes during spinal cord transection and regeneration, and correlations of nervous tissue stiffness with tissue microstructure. Strikingly, they observe that the stiffness of the spinal cord increases significantly in response to injury and returns to baseline after completion of repair. These results suggest that—contrary to our common intuition—neurite outgrowth, cell migration, and axonal regeneration are not compromised by alterations in the mechanical microenvironment.

At first glance, these findings are disappointing because they seem to suggest that mechanical factors fail to explain the difference between successful regeneration in zebrafish and limited repair in humans. However, on a more fundamental level, this study provides the first attempt to interpret the mechanisms that govern the cellular response during successful spinal cord repair and link the spinal cytoarchitecture before and after injury to changes in tissue stiffness. These efforts are significant in view of the recent recognition of the cellular mechanosensitivity in the nervous system (8) and the implications of neuromechanics on neural development and repair (9). Growing evidence suggests that many different cell types in the nervous system dynamically adapt their morphology, stiffness, protein expression, and biological function to changes in their mechanical environment (10). For example, throughout the past decade, numerous research groups have focused on probing stem cell differentiation and neuronal growth on substrates with varying stiffness, yet with mixed results: some studies found that soft environments promote neuronal growth, whereas rigid environments suppress neurite extension and neuron differentiation; others showed that neuronal outgrowth does not depend on the mechanical environment or even increases on stiffer substrates (8). Although the response to mechanical cues may vary between different cell

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*Correspondence: ekuhl@stanford.edu

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types, location, and time, we are increasingly recognizing the mechanosensitivity of neurons and glia within the nervous system (10). Admittedly, our understanding of neuronal mechanosensitivity is still in its infancy, but there is a general agreement that progress in this direction will be critical to understand, and possibly even modulate, the underlying mechanisms of development, disease, and repair in the central nervous system.

A key first step toward modulating the mechanical environment is characterizing regional variations in gray and white matter stiffness in the healthy state. Möllmert et al. (7) find that gray matter tissue is approximately twice as stiff as white matter both in the healthy baseline state and in sham operated animals. Their stiffness values are on the order of 60 Pa for gray and 30 Pa for white matter for spinal cord tissue slices of 300 μm thickness. Compared to the literature, these recordings are more than one order of magnitude smaller than the reported stiffness values for gray and white matter brain tissue that are typically on the order of kilopascals. For example, a recent indentation study reported values of 1.9 kPa for white and 1.4 kPa for gray matter for bovine brain tissue slices of 5 mm thickness, and a triaxial mechanical testing study found stiffness values of 4 kPa for gray and 2 kPa for white matter for human brain tissue cubes of 5 mm length (11). The range of the reported values varies hugely (8) and has been attributed mainly to differences in sample preparation, sample size, testing method, and tissue microstructure (12). However, the technique used throughout this study, AFM-guided nanoindenation, seems to be robust, reliable, and repeatable, with small variations within different groups and sufficient statistical significance between them.

A second important step toward understanding potential mechanisms of spinal cord regeneration is characterizing transient stiffness changes during spinal cord transection and regeneration. Möllmert et al. (7) find that 2 and 4 weeks after spinal cord transection, the white matter stiffness close to the lesion site doubles and reaches values comparable to their gray matter counterparts. Away from the lesion, white matter stiffness changes caudally, but not rostrally. Gray matter stiffness increases marginally but far less than white. Interestingly, 6 weeks postinjury, all stiffness values gradually begin to return to baseline. These findings are exciting for multiple reasons. First and foremost, they reinforce the notion that—unlike most other parts of our body—the central nervous system is capable of adjusting its mechanical environment in a dynamic and need-based fashion. With a baseline stiffness on the order of kilopascals or less, a small change in microstructure can have a large impact on spatial gradients and directionality, which, in turn, can alter transport phenomena and mechanical equilibrium (9). This makes mechanical signaling an incredibly efficient strategy of long-range communication (10). In spinal cord transection, it seems natural that alterations in mechanical stretch, forces, pressure, or surface tension promote the secretion of connective tissue growth factors, stimulate cell proliferation, and direct cell migration toward bridging the gap between the two sides of the injury. Interestingly, the observed transiently elevated stiffness does not create a mechanical barrier to obstruct axonal regrowth and penetration across the lesion site. This is probably the most translational aspect of this study because it suggests that there is no need to soften the mechanical environment to promote spinal cord repair in humans. Softening the glial scar to create a more stimulating environment for axonal regrowth has been proposed as a potential mechanism to trigger spinal cord regeneration (8). However, this study now suggests that successful spinal cord repair requires solutions that involve the collective engagement of both non-stiffness-related mechanical and biochemical factors.

Identifying relevant mechanical and biochemical factors and correlating nervous tissue stiffness to microstructure are important open questions with immediate applications in neuromechanics, neurodegeneration, and neuroprotection. The study by Möllmert et al. (7) makes a first attempt to explain dynamic stiffness changes by changes in microstructural composition including cell density, cellular connectivity, myelin content, and extracellular matrix composition. Unfortunately, in central nervous tissue, these correlations are far less one-to-one as they are in other hard and soft tissues. For example, we know that bone stiffness is strongly correlated with density and arterial stiffness increases with collagen content. Scientists are eager to identify similar correlations in the central nervous system, and cell density, myelin content, and axonal orientation all appear to be excellent candidates to explain mechanical stiffness (12). Yet, of these, myelin density seems to be the only microstructural determinant that can be confidently linked to nervous tissue stiffness (13). It is becoming increasingly clear that complex synergistic effects of single cell stiffness, cell density, cross-linking, vasculature, and extracellular matrix collectively contribute to modulate the mechanical signature of central nervous tissue. More multidisciplinary studies are needed to elucidate the interplay of chemical composition and mechanical properties and their implications for mechanosensing in the nervous system.

Taken together, despite the strong translational potential for improving treatment strategies in humans, we still know disappointingly little about the biochemomechanical signature of central nervous tissue during spinal cord injury. However, with the recent developments in traction force microscopy, micropillar arrays, atomic force microscopy, droplet inserts, and ferrofluidics, we now have the tools and technologies to characterize the biochemical and mechanical environment over multiple time and length scales both in vitro and in vivo. Quantitative spatiotemporal mappings of microstructure and stiffness in response to controlled spinal

cord injury in zebrafish provide a window into the interplay of biochemistry and mechanics during functional regeneration. A better understanding of the transient microenvironment during spinal cord repair could provide important cues to successfully rejuvenate, reroute, and regrow neuronal cells with the long-term vision to cure paralysis in humans.

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