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Novel biomaterials to study neural stem cell mechanobiology and improve cell-replacement therapies

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Abstract

Neural stem cells (NSCs) are a valuable cell source for tissue engineering, regenerative medicine, disease modeling, and drug screening applications. Analogous to other stem cells, NSCs are tightly regulated by their microenvironmental niche, and prior work utilizing NSCs as a model system with engineered biomaterials has offered valuable insights into how biophysical inputs can regulate stem cell proliferation, differentiation, and maturation. In this review, we highlight recent exciting studies with innovative material platforms that enable narrow stiffness gradients, mechanical stretching, temporal stiffness switching, and three-dimensional culture to study NSCs. These studies have significantly advanced our knowledge of how stem cells respond to an array of different biophysical inputs and the underlying mechanosensitive mechanisms. In addition, we discuss efforts to utilize engineered material scaffolds to improve NSC-based translational efforts and the importance of mechanobiology in tissue engineering applications.

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Introduction

Neural stem cells (NSCs) have been established as important mediators and effectors of plasticity, learning, and memory in the adult nervous system [1] and are envisioned as a potential source for transplantation in neurodegenerative diseases [2–4]. NSCs reside in two specific regions of the adult mammalian brain, the subventricular zone (SVZ) of the lateral ventricles and subgranular zone (SGZ) within the dentate gyrus of the hippocampus [1]. Hippocampal neural stem cells in particular have been indicated to play critical roles in learning, memory formation, behavioral regulation, and disease pathology, important processes that motivate a deeper basic understanding of NSC behavior [5]. Since the landmark discovery of these cells in mammals [6], the development of long-term NSC culture methods have enabled basic investigations of their behavioral regulation as well as exploration of their therapeutic potential to treat neurodegenerative disease, traumatic brain injury (TBI), spinal cord injury (SCI), and stroke [7].

NSCs, like other stem cells, are tightly regulated by the extracellular microenvironment within their resident tissues, collectively termed the stem cell niche [8]. Within these tissues, NSCs reside in proximity to more mature neural progenitor cell progeny, and it is possible to isolate and culture these populations. Throughout this review we will refer to cultured cells as “NSCs”, though a recognized caveat is that cultures may contain mixed populations. Previous research has revealed that biochemical cues present in the niche can strikingly direct NSC behavior *in vitro* and *in vivo* [9,10]. However, biophysical and specifically mechanical cues have been more recently implicated as a potentially important but relatively poorly understood signal input for NSCs [11]. Early *in vitro* work showed that softer 2D substrates that more closely emulate brain tissue stiffness (<1 kPa) promote neuronal differentiation of NSCs, whereas stiffer substrates (>1 kPa) suppress neurogenesis [12,13], of strong potential interest given the presence of tissue stiffness gradients within the hippocampus [14]. However, the mechanisms that govern this behavior are progressively being elucidated, and further studies are needed to confirm whether NSCs are similarly mechanosensitive in 3D, *in vivo* contexts. Furthermore, NSCs encounter and are regulated by many other types of mechanical input besides stiffness during development, injury, or disease. Therefore, it is

necessary to investigate impacts of these inputs is needed to assemble a more complete understanding of how biophysical cues regulate NSCs.

Within this field, biomaterials have not only played a role in enabling *in vitro* investigation of mechanobiology, but have also been harnessed as scaffolds to address common challenges in stem cell regenerative medicine such as inefficient expansion and differentiation, widespread death of transplanted cells, and limited homing to or retention in the desired site [15]. Scaffolds have a distinct advantage over the injection of dissociated "bolus" cell suspensions since it is possible to engineer "synthetic microenvironments" that support NSC survival and differentiation upon transplantation. Although scaffolds have shown promise in improving the engraftment of NSCs into the central nervous system (CNS), increased understanding of the mechanical effects of these scaffolds is again needed to enable precise tuning of NSC behavior [16].

In this review, we highlight recent studies in which innovative biomaterial systems have been engineered and exploited to further illuminate how NSCs respond to and process mechanical inputs. In addition, several relevant signaling mechanisms that respond to these material systems will be discussed, though a more thorough overview of NSC mechanotransductive pathways may be found elsewhere [17]. We begin by covering recent work describing novel effects of substrate stiffness and the impact of stretch stimuli on NSC fate commitment, neuronal maturation, and other cellular behaviors. We then discuss insights gleaned from a new generation of culture platforms that enable reversible tuning of substrate stiffness or incorporation of 3D architecture. Finally, we will discuss how engineered material systems are being used to improve translational strategies for neurodegenerative disease and neurological injury. While there have been important advancements in scaffold-based treatments in the spinal cord [18], this review will focus on recent efforts for application in the brain.

Biophysical regulation of NSCs

We and others have previously reported that substrate stiffness can specifically direct NSC fate commitment — soft substrates promote neurogenic differentiation of NSCs, while stiffer substrates suppress neurogenesis and increase astrocytic differentiation [13,19]. Moreover, we have identified Rho GTPase-mediated cytoskeletal dynamics and the transcriptional co-activator Yes-Associated Protein (YAP) as key players in stiffness-instructed NSC differentiation [20], and other work in the field has demonstrated the importance of focal adhesion proteins such as vinculin [21,22]. Adding to those findings, novel approaches have further explored the extent of NSC sensitivity to various biophysical inputs and the mechanisms that actuate mechanosensitive NSC behavior.

Substrate stiffness regulates NSCs through various intracellular signals

Although it is clear that substrate stiffness can strongly influence NSCs, many initial studies including some of our own [12] have primarily examined discrete stiffnesses that span orders of magnitude (e.g. 0.1−10 kPa). While such studies have clearly established that NSC proliferation and differentiation are mechanosensitive, the underlying experimental systems do not precisely elucidate the continuous, dynamic impact of stiffness on NSC behavior. Mosley et al. addressed this shortcoming by using a hydrogel that was engineered to have a continuous stiffness gradient [23]. Excising small circular portions down the length of this initial material generated gels with much finer relative differences in stiffness than those reliably achieved by conventional methods. Using these gels, they found that neurites from human induced pluripotent stem cell-derived NSCs cultured in neural differentiation media for 14 days were significantly longer on 0.9 kPa gels than on 1.44 kPa gels and that the expression of neuronal markers Tuj1 and MAP2 were affected by small differences in stiffness that could be missed in typical studies. Differences in neurite extension suggest differences in underlying cytoskeletal organization, which is a key mechanosensitive response in stem cells. Although many studies have identified broad regimes of stiffness that promote certain NSC behavior (e.g. stiffness <1 kPa as measured by shear rheology or AFM promotes neurogenesis [12,19,24]), this study strikingly implies that NSCs are much more finely tuned to stiffness cues than previously appreciated. An important general caveat with these studies is that various methods such as shear rheology or AFM are used to measure the elastic moduli of culture substrates, which does limit the extent to which measurements can be quantitatively compared between reports.

Previous studies have identified specific mechanosensitive molecules such as integrin and CD44 cell surface receptors that translate stiffness cues into intracellular signaling cascades in both stem and non-stem cells [25,26]. Continuing work has progressively revealed important new pathways that can direct stem cell fate commitment in response to stiffness input. For example, a growing body of evidence has indicated that intracellular calcium can regulate stem cell behavior [27,28] via mechanisms that are only starting to be investigated. In a recent study [29], Pathak and colleagues reported that Piezo1 (stretch-activated cationic channel) was expressed in human fetal-derived cortical NSCs and could induce spontaneous calcium influxes in a stiffness-dependent manner such that stiffer silicone elastomeric substrates (3.7 and 750 kPa) elicited greater Ca^{2+} activity compared to softer substrates (0.4 and 0.7 kPa). Notably, and contrary to earlier studies, the authors reported increased neuronal differentiation on stiffer substrates. They attributed this unexpected