Review

Engineering Biomaterials for Synthetic Neural Stem Cell Microenvironments

Lauren Little, Kevin E. Healy, and David Schaffer

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Engineering Biomaterials for Synthetic Neural Stem Cell Microenvironments

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1. Introduction

Neural stem cells (NSCs) have been isolated from various species—such as mice, rats, and humans—and from numerous regions in the developing and adult nervous system—including the subventricular zone (SVZ), the subgranular zone of the hippocampus, the cortical neuroepithelium, and the spinal cord.[1–8] In vivo, the NSC is encompassed by a microenvironment or niche that presents it with a repertoire of diffusible factors,[6,9,10] cell−cell interactions,[11,12] and extracellular matrix (ECM) ligands that bind to cellular receptors and thereby modulate signaling and gene expression (Figure 1).[13–15] Ultimately, these soluble and solid-phase components of the niche collectively regulate cell behavior and function—including mitosis, apoptosis, migration, and differentiation.[6,16–24]

NSCs have therapeutic potential to treat disorders and injuries such as Huntington's disease, multiple sclerosis, Parkinson's disease, stroke, and diseases and injuries of the spinal cord.[25–32] In cell transplantation therapies, NSCs have survived in various regions of the central nervous system (CNS), including the striatum, hippocampus, ventricles, SVZ, olfactory bulb, and cerebellum,[26,33–37] and have shown promising results when implanted at the injured/diseased sites in animal models for numerous diseases and injury, such as Sly disease, myelin degeneration, Parkinson's disease, and spinal cord injury.[33,34,38–41]

In general, successful novel cell transplantation-based therapies will hinge upon the ability to isolate stem cells, expand them in an undifferentiated state, induce their differentiation into a specific neuronal cell type or types, and engraft them in vivo in a manner that ensures their functional integration into the affected tissue. Each of these stages requires precise control over cellular behavior, which will therefore entail the successful development of systems that emulate the natural stem cell niche, that is, synthetic stem cell microenvironments. For example, ex vivo systems that support stem cell expansion and differentiation in a safe, scaleable, and economical fashion will be needed. In addition, in general, only a small fraction of stem cells or their progeny survive when implanted,[42–47] so there is a need to develop new systems or synthetic microenvironments that encourage successful incorporation, survival, and integration of NSCs into diseased or injured regions of the CNS.

Synthetic microenvironments have two major components: soluble and solid phases. For clinical applications, both components should be biochemically defined, reproducibly generated, nonimmunogenic[48] (and therefore human in origin), and not pose risks of pathogen transfer. The soluble phase, therefore, should avoid the use of serum, a poorly defined collection of hundreds of proteins and other components that can suffer from lot-to-lot variability. Fortunately, there has been considerable progress in identifying and utilizing defined soluble factors to modulate stem cell behavior, leading to the development of defined serum-free media for culturing human embryonic stem cells. As these important advances have been discussed elsewhere,[49–54] this review will focus on the solid phase, specifically on the development of various materials for NSC culture including natural, semisynthetic, and fully synthetic materials.

Although ECM molecules are a major component of the cellular niche, exploiting these molecules to construct controlled stem cell microenvironments has been comparatively difficult because they are extremely large (e.g., laminin is 850000 Da), have multiple isoforms and glycoforms, are difficult to purify to homogeneity, and may be difficult to obtain from large-scale and high-quality sources. However, for clinical applications, matrices or substrates used for stem cell culture or implantation must satisfy many of the same criteria as soluble components. That is, they should be biochemically well-defined, purified to near or complete homogeneity, be bioactive via the presentation of key regulatory signals, nontoxic, nonimmunogenic, and not pose risks of pathogen transfer. In addition, just as serum adds a complex mixture of poorly defined components to the soluble medium, serum proteins can also adsorb onto cell culture

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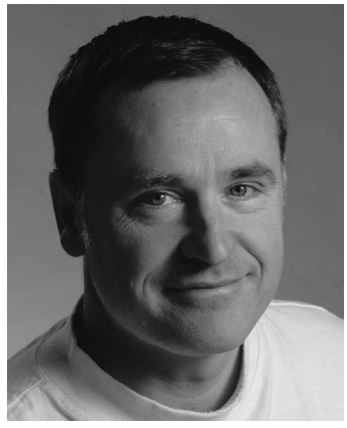

Kevin E. Healy, Ph.D., is a Professor at the University of California at Berkeley in the Departments of Bioengineering and Materials Science and Engineering. He received a Bachelor of Science degree from the University of Rochester in Chemical Engineering in 1983. In 1985, he received a Masters of Science degree in Bioengineering from the University of Pennsylvania, and in 1990, he received a Ph.D. in Bioengineering, also from the University of Pennsylvania. He was elected a Fellow of the American Institute of Medical and Biological Engineering in 2001. He has authored or co-authored more than 200 published articles, abstracts, or book chapters which emphasize the relationship between materials and the tissues they contact. His research interests include the design and synthesis of biomimetic materials that actively direct the fate of mammalian cells and facilitate regeneration of damaged tissues and organs. Major discoveries from his laboratory have centered on the control of cell fate and tissue formation in contract with materials that are tunable in both their biological content and mechanical properties. These materials find applications in medicine, dentistry, and biotechnology.

surfaces and thus complicate the development of fully defined soluble and solid-phase systems for stem cell culture.

An increasingly employed approach for emulating the ECM involves identifying bioactive motifs present in these molecules and grafting synthetic analogues of these signals onto a material. For example, cells engage with ECM ligands via receptors such as integrins, a major family of heterodimeric adhesion cell receptors, composed of α and β subunits, whose downstream signaling can regulate growth, differentiation, and survival.[17] Integrins are known to bind to several common polypeptide motifs such as arginine−glycine−aspartic acid (RGD),[55,56] and chemically synthesized peptides containing this signal have been broadly used in biomaterials engineering, as discussed below.[57,58]

David Schaffer is a Professor of Chemical Engineering, Bioengineering, and Neuroscience at the University of California, Berkeley, where he also serves as the Associate Director of the Berkeley Stem Cell Center. He graduated from Stanford University with a B.S. degree in Chemical Engineering in 1993. Afterward, he attended Massachusetts Institute of Technology and earned his Ph.D. also in Chemical Engineering in 1998 with Professor Doug Lauffenburger. While at M.I.T., Dave minored in Molecular and Cell Biology. Finally, he did a postdoctoral fellowship in the laboratory of Fred Gage at the Salk Institute for Biological Studies in La Jolla, CA, before moving to UC Berkeley in 1999. At Berkeley, Dr. Schaffer applies engineering principles to enhance stem cell and gene therapy approaches for neuroregeneration. This work includes mechanistic investigation of stem cell control, as well as molecular evolution and engineering of viral gene delivery vehicles. David Schaffer has received an NSF CAREER Award, Office of Naval Research Young Investigator Award, Whitaker Foundation Young Investigator Award, and has been named a Technology Review Top 100 Innovator. He was also awarded the American Chemical Society BIOT Division Young Investigator Award in 2006 and the Biomedical Engineering Society Rita Shaffer Young Investigator Award in 2000.

In addition, it is becoming increasingly clear that not only the biochemical but also the mechanical properties of the microenvironment can modulate the cytoskeleton, the adhesion and growth of cells, and even the differentiation of stem cells;[59–61] therefore, it would be desirable to be able to finely tune the mechanical properties of the culture system. Collectively, the biochemical and mechanical signals of proteins or materials mimicking the solid phase of the native stem cell microenvironment will play a major role in controlling first the expansion and then the differentiation of stem cells for clinical applications.

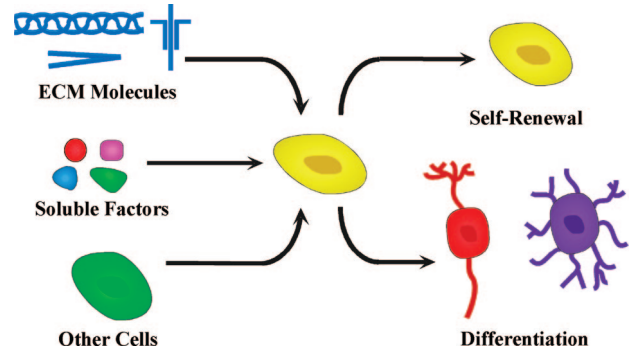

Figure 1. Influencing components in the stem cell microenvironment. Stem cells are influenced by many components of their microenvironment including ECM molecules, soluble factors, and other cells. The combination of all of these signals determines whether the cell undergoes self-renewal or differentiation.

Table 1. References That Are Discussed in This Review, Organized by Class of Material Utilized in Each Study[a]

type of surface	species	stage of develop-ment	location of NSCs	medium	ref
natural	rat	E	cortex	serum-free	73
	rat	E	cortex	serum-free	72
	rat	E	cortex	serum-free	71
	rat	E	cortex	serum-free	70
	rat	A	SVZ	serum-free	76
	mouse	E	cortex	serum-free	80
	human	N	cortex	serum-free	
	human	F	cortex	serum-free	81
	rat	F	striatum	serum-free	82
	rat	A	hippocampus	serum-free	84
	mouse	E	cortex	serum-free	
	rat	A	hippocampus	serum-free	85
	mouse	E	hippocampus	serum-free	88
	rat	A	spinal cord	serum	89
	rat	A	hippocampus	serum-free	90
semisynthetic	rat	E	cortex	serum	91
	rat	E	forebrain	serum-free	99
	rat	F	NR	serum-free	100
synthetic	rat	A	hippocampus	serum-free	106
	mouse	E	cortex	serum-free	103
	mouse	A	NR	NR	115
	rat	A	hippocampus	serum	119
	rat	E	cortex	serum-free	122
	mouse	N	cerebellum	serum	120
	rat	E	cortex	serum-free	12
	rat	E	cortex	serum-free	135
	rat	A	hippocampus	serum-free	139
	rat	F	hippocampus	serum-free	150
	rat	A	spinal cord	serum	152
	mouse	N	cerebellum	serum	153
	mouse	N	cerebellum	serum	149

[a] NSCs and NPCs isolated from various locations in the CNS of rats, mice, and humans—and from various stages of development—were used in these studies. Finally, given the strong influence that serum can exert on both the soluble and the substrate components of the cellular microenvironment, the type of medium utilized is listed. NR, not reported; E, embryonic; F, fetal; N, neonatal, and A, adult.

2. In Vitro Studies

Although cells that can be expanded in vitro and undergo multipotent differentiation into neurons and/or glial cells have been isolated from numerous regions of an organism,[62] this review will focus on stem cells isolated directly from the CNS (Table 1). The terms "neural stem cell", "neural progenitor", and "neural precursor" have often been used interchangeably in the literature. We will use the term "neural stem cell" to refer to a population of cells with the capacity for extended self-renewal or proliferation in an immature state, as well as multipotent differentiation into neurons and glial cells. In addition, the term neural progenitors or precursors (NPCs) refers to cells that exhibit multipotent differentiation but only have a more limited capacity for self-renewal. All of these cell populations can be grown either as neurospheres—cell aggregates in suspension—or as an adherent monolayer.

Extensive in vitro studies have developed two-dimensional surfaces or three-dimensional (3D) gels for culturing either relatively uniform NSC populations or to a lesser extent CNS tissue explants. In particular, these efforts have focused on engineering substrates, sometimes in conjunction with growth or other soluble factors, that support or regulate specific cellular behaviors such as proliferation, differentiation into either neurons or glia, or neurite growth from neurospheres. The development of materials for in vitro cell culture is important for stem cell expansion and differentiation and can also serve as a first step toward the design of materials that can support the survival and engraftment of stem cells in vivo upon implantation.

2.1. Natural Surfaces and Gels

Numerous surfaces and gels have been generated from natural components such as collagen, other ECM proteins, and calcium alginate. These materials contain native biochemical signals and have enabled the attachment and expansion of many other cell types,[63–66] perhaps related to the fact that several ECM molecules including fibronectin, laminin, thrombospondin, and collagen IV are known to be present in close proximity to NSCs in vivo.[67–69] However, natural components can face several challenges. For example, it can be difficult to tune the mechanical properties of natural materials, and it is generally not possible to independently tune the mechanical and biochemical signals of these systems. Natural components, such as ECM proteins, also have problems with purity and the availability of large-scale sources of the materials, particularly if human proteins are involved. Regardless, studies with these natural materials provide highly valuable information and aid in the elucidation of design criteria for synthetic cellular microenvironments.

2.1.1. Collagen

Collagen, a triple helix protein that accounts for approximately 30% of all protein found in vertebrate animals, is present in skin, connective tissue, and many other regions throughout the body.[63] There are at least 21 types of collagen with varying biochemical and physical properties that comprise many distinct structures ranging from cornea to cartilage. Type I collagen is predominantly found in skin, bone, and tendon, where larger forces are exerted, suggesting a role in the mechanical integrity of tissue.[63]

Numerous efforts have used 3D type I collagen, which can form gels, to culture rat embryonic cortical NSCs.[70–73] In one study, O'Conner et al.[73] cultured neurospheres on the top of collagen I gels and found that cells were able to migrate and disperse from the spheres and subsequently extend neurite processes. Cells that migrated in the first 10 days were primarily neurons, while later migrating cells were primarily glial cells.[73] In a subsequent study, neurons in these gels were able to form networks exhibiting synaptic transmission with the neurotransmitter GABA.[72]

Ma et al.[71] further explored the use of the collagen I gels with embryonic cortical NSCs. Most cells remained attached to and proliferated on the gel surface during the first week of culture, and the cells that did differentiate during this initial time gave rise primarily to neurons that showed the capacity to form synapses. During the second week of culture, however, the remaining NSCs differentiated into glial cells.[71] In addition, two-dimensional gels show that collagen supported cell attachment and culture, but 3D gels may better mimic the geometry experienced in vivo. Therefore, cells have been added to a collagen I solution, which was then allowed to gel to create a 3D system. Many of the neurospheres in the resulting 3D gels contained high levels of dead cells due to limited nutrient and oxygen transport, but cell viability was improved by using a rotating wall vessel (RWV) reactor.[70] Cells were first seeded into collagen I gels, and the gels were then placed into the reactor. The rotating wall reactor allowed cells to create tissuelike structures with differentiated neurons and astrocytes intermingling in the middle of the gel and NSCs closer to the surface.[74,75] These

studies utilizing collagen gels show the promise of using a 3D environment to create complex structures of differentiated cells.

2.1.2. Other ECM Molecules

ECM molecules other than collagen have also been used to prepare surfaces for the culture and differentiation of NSCs. For example, Matrigel is a complex mixture of laminin, collagen IV, and heparan sulfate,[49,76] whereas E-C-L attachment matrix is a combination of entactin, collagen IV, and laminin. Both Matrigel and E-C-L, as well as single ECM molecules other than collagen, have been extracted from animal sources and used to create culture microenvironments in vitro. In addition, soluble factors have been tested in conjunction with these various mixtures of ECM proteins.

Whittemore et al.[76] explored the effects of combinations of ECM and growth factors on adult rat SVZ NPC propagation.[76] While epidermal growth factor (EGF)-treated NPCs attached to uncoated polystyrene (PS) plates and plates coated with E-C-L, laminin, and fibronectin, the cells did not expand on any of these surfaces. By contrast, NPCs grown with fibroblast growth factor-2 (FGF-2) attached to and proliferated on all surfaces except PS. Finally, NPCs exposed to FGF-2 plus heparin—which aids in FGF-2 signaling by binding to both FGF-2 and its receptor[77–79]—formed nonadherent neurospheres on plastic and attached as a monolayer to the remaining surfaces.[76] Collectively, these results demonstrated that precursor cells propagated with the same mitogen can exhibit a different behavior as a function of the substrate.

Neurospheres of postnatal human cortical NSCs and mouse embryonic cortical NSCs have been analyzed on various ECM proteins adsorbed to glass surfaces.[80] NSCs migrated from neurospheres seeded on the various surfaces, with more migration observed on laminin and Matrigel than on fibronectin or poly-L-ornithine. To stimulate cell differentiation, cultures were exposed to brain-derived neurotrophic factor (BDNF), fetal bovine serum, FGF, and all-*trans*-retinoic acid. A larger number of astrocytes and neurons differentiated from NSCs on laminin and Matrigel; however, longer neurite growth was observed on fibronectin. Additionally, the α_6 integrin was also shown to be functionally important for cell attachment to laminin.[80] Once again, this study showed the importance of tuning the mixture of soluble factors and substrates to elicit specific cellular behaviors.

These studies are examples of the fact that ECM and other factors combine to regulate cell behavior, which raises the experimental difficulty of exploring many possible combinations of factors. To address this challenge, cellular microarrays, in which cells are plated on an array of "printed" features or islands of ECM and/or soluble factors, are powerful tools to test many combinations of signaling factors in parallel. A combinatorial microarray of ECM, growth factors, and morphogens was developed to analyze synergistic effects in regulating human fetal cortical NSCs function.[81] The cortical NSCs were able to form 3D weblike structures on fibronectin but only grew in a monolayer on laminin, vitronectin, and Matrigel. Laminin and soluble Wnt3A encouraged neuronal fate, while transforming growth factor-β (TGF-β) and bone morphogenic protein-4 (BMP-4) drove glial differentiation.[81] This important study demonstrated that in constructing a microenvironment to regulate cell function, synergistic effects of signaling factors on cell behavior may be difficult to predict based on the effects of each individual component. However, complex combinations of factors, including ECM, may be necessary to achieve tight control over cell function.

Nakajima et al.[82] developed cellular microarrays that analyzed various ECM components, ProNectin F or ProNectin L, and different growth factors. ProNectin F and ProNectin L are recombinant proteins that form β-sheets displaying an RGD sequence from fibronectin or an IKVAV sequence from laminin at the ends of the intervening loops, respectively.[82,83] Rat fetal striatal NPCs adhered well to fibronectin, laminin, ProNectin L, and Pronectin F but not to features/spots with just growth factors. Conditions that included EGF elicited higher proliferation rates and cellular expression levels of the intermediate filament protein nestin, a marker for NPCs. In addition, more cells differentiated into neurons on fibronectin and ProNectin L, while more NPCs differentiated into astrocytes on ProNectin F and laminin.[82] This study once again demonstrated that microarrays can yield substantial information on the combinatorial effects of substrate and soluble factors on cell function, results that will aid the development of bioactive, synthetic microenvironments.

A cellular microarray has also recently been developed for functional genomics screening in NSCs.[84] The microarray was synthesized via patterning a gold surface with a poly(dimethyl siloxane) (PDMS) stamp coated with 11-mercaptoundecanoic acid and then immersing the "stamped" surface in tri(ethylene glycol)undecane-thiol. This synthesis resulted in a surface with small regions where cells could attach, surrounded by regions containing ethylene glycol that resist cell attachment. When cells were seeded at a low density along with laminin on the microarrays, the resulting clonal populations of NSCs could be analyzed for any number of cell behaviors, including survival, proliferation, intracellular signaling, and differentiation.[84] NSCs were infected with viral vector carrying a cDNA library, and the cellular microarray was used to screen the resulting NSC population for cDNA clones that enabled cell proliferation in low growth factor concentrations.[84] This study demonstrates that a variety of functional genomic screens can be implemented on high-throughput microarrays for gene discovery. The implementation of such high throughput gene function screens on cellular microarrays coated with a variety of ECM molecules could further elucidate connections between ECM-related signaling and cell behavior.

In addition to high-throughput screens, surface patterning can be used to analyze the effects of spatially organized signaling factors on cellular behavior. For example, adult rat hippocampal NSCs have been cultured on laminin-coated surfaces that were first patterned via photolithography on silicon wafers and then transferred to PS via solvent casting. The surfaces exhibited parallel strips of alternating heights that were 13 μm wide, 4 μm high, and 16 μm apart.[85] While NSCs that were differentiated on unpatterned surfaces had randomly aligned processes, the surfaces with parallel wells yielded differentiated neurons with processes aligned along the direction of the grooves. When cocultured with astrocytes on these surfaces, NSCs extended processes along the cytoskeletal filaments of the astrocytes, while the astrocytes spanned and thereby made contact with neurons on different grooves. Furthermore, prior studies have shown that astrocytes can promote neurogenesis.[86,87] In the cocultures of astrocytes and NSCs on these patterned substrata, more of the NSCs differentiated into neurons, which in turn exhibited

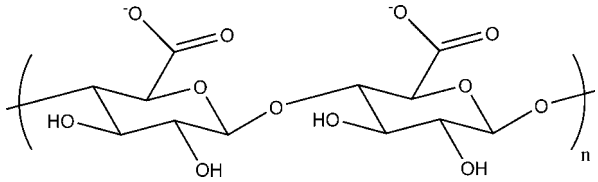

Figure 2. Chemical structure of the natural polymer alginate.

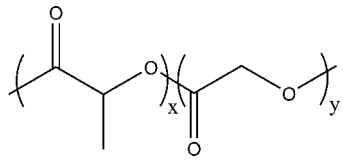

Figure 3. Chemical structure of the repeating unit for the copolymer poly(lactic-co-glycolic acid) (PLGA).

longer processes.[85] This study demonstrated the effects of both substrate patterning and cell cocultures on cell differentiation and alignment, findings that could potentially be applied toward the assembly of implantable neural prostheses and cell-based devices.

2.1.3. Calcium Alginate

Alginates, polyanionic polysaccharides that are isolated from brown sea algae and contain mannuronic and guluronic acids (Figure 2), gel in the presence of bivalent cations such as calcium and barium.[64–66] Because alginates are both biocompatible and inexpensive, they have been broadly explored in cell encapsulation and tissue-engineering applications.[64] Recently, Li et al. encapsulated mouse embryonic hippocampal NPCs in calcium alginate microcapsules. The cells proliferated and maintained nestin expression along with the ability to differentiate into neurons and glial cells.[88]

In another study, rat adult spinal cord NSCs expressing green fluorescent protein (GFP) were grown in 3D calcium alginate gels with capillary channel features, which were formed by the oriented diffusion of copper ions during gel formation prior to the addition of cells. This geometry was designed to promote directional axonal growth through a site of injury and thereby aid axon regeneration.[89] ECM proteins adsorbed on the gels did not significantly change the density of axons or the length of axon ingrowth into the channels as compared to channels without ECM proteins. To assess the performance of the biomaterial in an organotypic culture, NSCs were seeded in calcium alginate gels for 7 days in serum-containing medium and then transplanted into the region between the entorhinal cortex and the hippocampus in rat brain slice cultures. The resulting brain slices exhibited GFP-expressing glial cells and neurons with axons aligning along the capillary features of the gel.[89]

Another system developed by Ashton et al.[90] explored alginate hydrogels, embedded with poly(lactide-co-glycolide) PLGA (Figure 3) microspheres containing the enzyme alginate lyase, for NSCs transplantation. Because alginate hydrogels are not naturally degraded enzymatically in vivo in mammals, the addition of encapsulated alginate lyase allows for the controlled degradation of the alginate hydrogel.[90] Without the lyase, the NSCs exhibited elongated processes, while gels with the encapsulated lyase supported proliferating NSC neurospheres. These studies show the potential of calcium alginate for engineering microenvironments for NSCs. Furthermore, these results indicate that when encapsulated in some materials, cells can presumably provide their own signals and therefore do not require the addition of ECM molecules, although adding exogenous signals may afford more control over cell behavior.

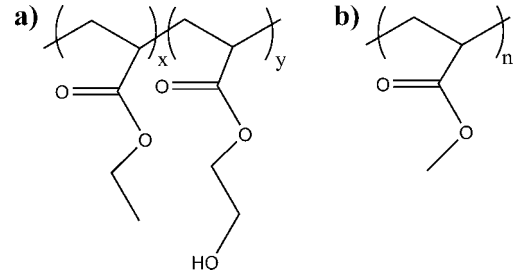

Figure 4. Chemical structure of repeating units of (a) poly(ethyl acrylate)-co-poly(hydroxyethyl acrylate) and (b) poly(methyl acrylate).

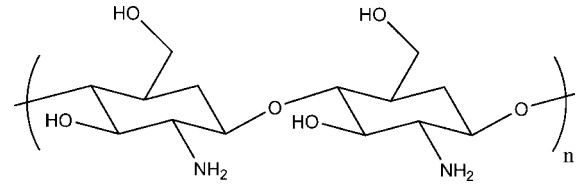

Figure 5. Chemical structure of chitosan, a natural polymer derived from chitin.

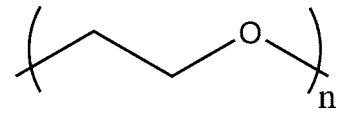

Figure 6. Chemical structure of the repeating unit of PEG.

2.2. Semisynthetic Surfaces and Gels

Surfaces and gels have also been developed using a blend of synthetic and natural components. The natural component in these blends is typically an ECM protein that is adsorbed to the synthetic component and presents signals to modulate cell attachment, growth, and differentiation. Moreover, the addition of a synthetic component enables control over the architecture and mechanics of the materials. These bioactive, modular materials can therefore be viewed as an intermediate step toward developing completely synthetic materials, although the ECM protein still poses challenges for purity, immunogenicity, scaleability, and other considerations.

Soria et al.[91] tested the behavior of rat embryonic neural explants from the medial ganglionic eminence and ventricular zone of the cerebral cortex on various hydrophobic and hydrophilic polymers coated with laminin. The polymers that best supported cell adhesion and differentiation were poly(ethyl acrylate)-co-poly(hydroxyethyl acrylate) (Figure 4a), poly(methyl acrylate) (Figure 4b), and chitosan, which are polymers with an intermediate degree of hydrophobicity. Chitosan is a *N*-deacetylated derivative of chitin, a component in the exoskeletons of many insects (Figure 5).[92,93] All three materials exhibited differentiated neurons and glial cells, although it was not clear whether these mature cells arose from precursor cells or were already present in the explant. Importantly, the adsorbed layer of laminin was necessary for successful explant culture, and the conformation of the laminin adsorbed on each polymer was likely a key factor in the relative success of each material, as the orientation of ECM proteins can greatly affect cellular behavior, including attachment and proliferation.[91,94–98]

Other ECM proteins such as collagen have also been used in conjunction with a synthetic polymer. Rat embryonic

forebrain NPCs were cultured on a triblock copolymer, consisting of poly(ethylene glycol) (PEG) (Figure 6) flanked by poly(lactic acid) blocks.[99] NPCs grown on this material in the presence of FGF-2 exhibited increased cell growth, although the addition of collagen only to the cell suspension prior to cell plating had no real effect, indicating that collagen does not exhibit an effect without FGF. When FGF-2 and collagen were used in combination, apoptosis decreased and metabolism increased, although the total number of cells and the relative level of β-tubulin content did not significantly change as compared to when no FGF-2 or collagen was used.[99] Finally, FGF-2 and collagen conditions produced neurons with longer processes as compared to the other conditions with either FGF-2 or collagen.

As a final example of a semisynthetic material employing a non-ECM protein, a mixed self-assembled monolayer (SAM) composed of 16-mercapto-1-hexadecanoic acid and (1-mercaptoundec-11-yl) triethylene glycol (TEG) thiol was generated on gold. After the carboxylic acid of the SAM was esterified, Ni-NTA was chelated to the surface followed by the addition of a recombinant hexahistidine-tagged EGF.[100,101] Although cell attachment was initially weak, cells attached specifically to the surface via the EGF receptor (EGFR), as the addition of soluble EGF blocked attachment.[100] Fetal NPCs could be maintained on the surface for five days and retain their multipotency, as they were still able to differentiate into both neurons and glia. This maintenance may be related to previous work showing that EGFR and nestin expression can be correlated.[102] These studies collectively demonstrate that natural components can provide biochemical signals necessary to support cell attachment, proliferation, and differentiation when presented from a synthetic substrate. Promising semisynthetic materials also provide a promising basis for the development of fully synthetic materials that avoid some challenges of using isolated proteins, as these can potentially be replaced with recombinant or synthetic signals.

2.3. Fully Synthetic Surfaces and Gels

Natural ECM proteins offer the important advantage of presenting both identified and likely unidentified motifs that bind to cellular receptors and thereby regulate cell behavior. However, natural components have the potential to elicit an immune response if implanted, can transfer immunogenic molecules to stem cells,[48] can pose a risk of pathogen transfer, and often do not offer the capacity to readily control the mechanical properties of the material. By comparison, materials composed of primarily synthetic components offer advantages including low immunogenicity, reproducible and scaleable synthesis, and the ability to tune mechanical and biochemical properties, an important consideration for stem cells.[59–61] However, biofunctionalizing synthetic materials to present signals to support cell survival, proliferation, and differentiation can be challenging and may involve elaborate synthesis or conjugation schemes. Furthermore, it can be difficult to generate synthetic analogues of complex bioactive motifs, particularly when the intricate signals present within large ECM proteins may not be fully characterized.

2.3.1. Self-Assembling Peptides and Peptide Amphiphiles

Specific polypeptide sequences have the capacity to self-assemble into various structures, ranging from assembly of β-sheets via hydrogen bonding to cylindrical micelles via hydrophobic interactions.[83,103,104] To build upon these capabilities for creating bioactive matrices, the self-assembling peptide sequences can be synthesized as fusions to motifs found in ECM proteins, including RGD and IKVAV from fibronectin and laminin,[56,105] respectively, to create self-assembled structures that can engage cellular adhesion receptors. These synthetic peptides also offer the advantage of being able to display a broad diversity of natural and even unnatural side chains from the peptide backbone, enabling the creation of multifunctional assemblies.

One example of such a self-assembling material is a triblock protein containing an RGDS motif.[106] The protein is designed with a random coil region flanked by two identical amphiphilic leucine zipper sequences that allow for the formation of helices that can multimerize with the termini of other copies of the polypeptide, allowing for self-assembly into a gel.[107–109] Incorporation of the RGDs into the random coil region allowed for better adhesion of individual adult rat hippocampal NSCs, while cells formed nonadherent neurospheres on surfaces of gels lacking the RGD motif.[106] Silva et al.[103] developed an IKVAV-containing peptide amphiphile unit that self-assembles into micelle nanofibers, via hydrophobic forces, for use with E13 mouse embryonic cortical neuronal precursor cells.[103,110,111] In nanofiber scaffolds, the neuronal precursors differentiated into neurons with extensive processes, while very few cells differentiated into astrocytes.[103,112–114]

Another self-assembling peptide system has been developed using motifs from ECM proteins, such as YIGSR, RGDS, and IKVAV, and bone-homing peptides.[104,115–117] The latter had previously been isolated via in vivo phage display, in which a phage library is injected into animals for the identification and isolation of displayed peptides that mediate viral localization to a specific tissue, in this case bone marrow. The bone-homing peptides were employed based on reports that bone marrow-derived cells could differentiate into neuronlike cells.[118] Peptides composed of self-assembly domains fused to bioactive motifs and formed a 3D fibrous structure driven by β-sheet formation in the presence of salt, similar to structures seen via scanning electron microscopy with Matrigel.[104,116,117] NPCs were seeded on top of and subsequently infiltrated into the scaffold. The cells differentiated into neurons and astrocytes on all functionalized peptide networks, and the highest cell viability was observed on the self-assembling peptides with the bone-homing peptides.[115]

A study using peptides that assemble into fibrous structures via β-sheet formation showed that this scaffold encouraged putative neural stem or precursor cells from adult rat hippocampal slices to migrate away from tissue explants laid on top of the scaffold.[119] Cells expressing nestin, as well as larger number of neurons and glia, were found in the scaffold following this migration. In addition, the cells that infiltrated into the scaffold could subsequently be recovered and cultured on laminin-coated plates.[119] This system could therefore represent a useful method for extracting stem cells from tissue slices, although additional characterization will be required to validate that the isolated cells are true stem cells, that is, capable of self-renewal and multipotent differentiation.

Collectively, these self-assembling materials represent a unique set of building blocks that form complex structures in conjunction with presenting biochemical polypeptide signals similar to the ECM molecules mentioned earlier.

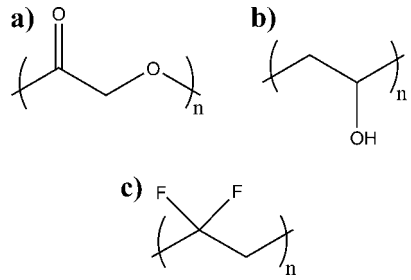

Figure 7. Chemical structure of the repeating unit for (a) poly(L-lactic acid) (PLLA), (b) poly(vinyl alcohol) (PVA), and (c) poly(vinylidene fluoride) (PVDF).

These highly modular systems can be designed to incorporate multiple biochemical signals, with peptides that assemble into other complex geometries or into structures whose mechanical properties can be tuned.

2.3.2. Synthetic Polymers

NSCs have also been cultured on numerous synthetic polymers, many of which have previously been used with other cell types for many applications including tissue engineering and controlled drug delivery.[12,120,121] Optimizing these materials may lead to the development of reproducible, scalable, nontoxic, and nonimmunogenic materials for in vitro expansion or differentiation, as well as in vivo implantation, of NSCs.

Wang et al.[122] used PS surfaces coated with synthetic poly-D-lysine or lysine-alanine sequential (LAS) polymers for culturing rat embryonic cortical NSCs.[122–124] The LAS copolymers were designed with repeating units of lysine-alanine to form an ordered copolymer chain.[123,124] When a low density of neurospheres was plated on these surfaces, cells attached and extended processes that were sufficiently long to interconnect the spheres. However, when higher numbers of neurospheres were seeded on the surfaces, cells migrated from the spheres and differentiated into astrocytes but did not form processes to connect the spheres.[122] Analogous distinct behavior as a function of cell density has been observed in other studies with neurons and NSCs.[125–127]

Electrospinning is a technique that applies a strong electric field across an extruding polymer solution to greatly elongate the solution stream, thereby depositing thin filaments of polymer onto an underlying surface. To create a fiber network, a spinning disk is used as the collection surface, and a dense nanofiber mat is created from the single fiber. The fiber dimension and size, as well as the polymer composition, can be precisely controlled.[128] Poly(L-lactic acid) (PLLA) (Figure 7a) has been used previously for nerve tissue regeneration because of its biocompatibility and biodegradability.[129] To extend upon these capabilities, mouse neonatal cerebellar NPCs were cultured on electrospun PLLA nano- and microfiber scaffolds and subsequently differentiated into cells with neurites aligned with the fibers. Importantly, cells exhibited longer neurites on nanofibers than on microfibers,[120] demonstrating that the size and topology of scaffold features can modulate cell differentiation and morphology.

Young et al.[12] studied rat embryonic cortical NSC behavior on the hydrophobic polymer polyvinyl alcohol (PVA) (Figure 7b) as well as amphiphilic poly(ethylene-co-vinyl alcohol) (EVAL) surfaces,[12,130,131] based in part upon prior work with differentiated cortical neuronal cultures on EVAL.[132–134] Cells, either as single cells or as neurospheres grown in serum-free medium, were not able to attach or survive on the PVA surface, while single cells attached to but did not proliferate on the EVAL. In addition, neurospheres at lower density attached to EVAL surfaces and extended neurites between the spheres, while a higher density of neurospheres did not attach to the surface or differentiate.[12] This study again shows the important effect of biomaterial chemistry—specifically the relative hydrophobicity of the polymer—along with cell density, on cellular behavior.

Rat embryonic cortical NSCs have also been grown in serum-free medium on PVDF (Figure 7c) and chitosan surfaces.[135] The PVDF material has previously been used as a biomaterial to aid in nerve regeneration, in part due to its mechanical strength.[136–138] On both surfaces, cells within neurospheres extended processes between the spheres, with shorter processes formed on PVDF.[135] PVDF surfaces also biased cell differentiation toward astrocytes, while chitosan surfaces favored a neuronal fate. Finally, a population of proliferating cells was maintained on PVDF but not on chitosan.[135] Future work may elucidate the chemical differences between these surfaces that elicit this distinct behavior.

Biofunctionalized interpenetrating polymer networks (IPN) have also been used to culture stem cells.[139] This IPN is composed of polyacrylamide interpenetrated with a PEG and poly(acrylic acid) network.[140,141] The IPN modulus can be controlled by tuning the amount of cross-linker incorporated into the polyacrylamide network, and biochemical signals can be grafted to polymer termini in the PEG network, which also prevents nonspecific protein adsorption. Importantly, the mechanics and chemical signals can therefore be tuned independently to create a nonfouling surface.[142] For example, the material has been functionalized with a number of synthetic peptides and even recombinant proteins.[143] When presenting a peptide motif derived from bone sialoprotein, bsp-RGD(15), the surface could support either the proliferation or the differentiation of NSCs in serum-free medium, depending on the soluble media conditions.[139] This system is therefore the first fully chemically and biochemically defined NSC culture system.

In summary, fully synthetic, biofunctionalized materials can support cell proliferation, and the addition of differentiating media leads to multipotent differentiation. Future work may explore the extent to which the substrate can guide cell lineage commitment. Furthermore, the use of thick gels can enable studies of the effects of matrix mechanics on NSC proliferation and differentiation (Saha et al., submitted for publication).

3. In Vivo Studies

There have been extensive efforts to implant neural stem or progenitor cells for neural repair in the absence of a scaffold.[144–146] Previous studies without materials have shown that the implanted cells can aid in tissue repair via several mechanisms, including paracrine delivery of therapeutic molecules, thereby reducing the toxic nature of the microenvironment, and in some cases potential functional cellular integration into host tissue.[147–149] However, a major problem is that generally only a small fraction of stem cells or their differentiated progeny survives when implanted, often due to inflammation and hypoxia present at the site of injury or disease.[44,45,47,147] Current methods of scaffold synthesis can allow for considerable control over the cellular microenvironment, which can serve as the basis of implantable materials to enhance the survival of engrafted cells.

Wu et al.[150] implanted rat fetal hippocampal NPCs encapsulated in an alginate gel into the spinal cords of rats, as this material has supported the growth and survival of NPCs in vitro and in vivo.[150,151] When monolayer-grown cells were implanted, there was not successful incorporation into host tissue. However, when neurosphere-grown NPCs were dissociated, incorporated into an alginate sponge, and implanted, the cells integrated well into the host tissue and extended processes into the surrounding tissue. Most cells differentiated into astrocytes, with some neurons.[150]

Another study implanted a mesh of poly(glycolic acid) (PGA)—which was saturated with pluronic F127 and covered with a hydroxymethylcellulose membrane—into a transected spine.[152] When the material was coimplanted with rat adult spinal cord NSCs, the resulting graft developed neurons along with some astrocytes, and axons from the NSC-derived neurons were able to extend beyond the area of injury and potentially help the injured animals regain partial coordinated use of their hind limbs 4 weeks postsurgery. Animals implanted with just the polymer scaffold regained some use of their hind limbs, but the movements were not coordinated, as seen in the animal group implanted with scaffold and cells. By contrast, animals implanted without scaffold had the formation of scar tissue and astrocytes surrounding the scar, with very little behavioral improvement.[152]

A unique scaffold using two synthetic material layers was analyzed for the ability to aid the regeneration of injured spinal cord. Both layers were made of PLGA and a block copolymer of PLGA-polylysine; however, one was seeded with murine neonatal cerebellar NPCs, whereas the other contained long axially aligned pores to allow for axonal guidance.[153] The bilayer material was implanted such that the first layer lay against the exposed gray matter in a midline lateral hemisection of the spinal cord. The scaffold, with and without cells, mediated recovery of hindlimb function, although using both cells and scaffold mediated the best overall improvement. The scaffold also allowed for axon extension beyond the site of implantation.[153] This work thus successfully used a combination of polymer chemistry and macroscale structure to yield a therapeutic result.

Park et al.[149] explored the implantation of a scaffold made of woven PGA fibers, which had been used previously as a transplant scaffold for cartilage repair, with neonatal murine cerebellar NPCs into mice with hypoxic-ischemic injury.[149,154] On this scaffold in vitro, the NPCs spontaneously differentiated into both neurons and glia that extended processes to wrap around several PGA fibers. After 4 days of culture and subsequent implantation, this scaffold exhibited differentiated neurons and glial cells and even host neuron and oligodendrocyte cell infiltration into the scaffold. After degradation of the biodegradable scaffold, vascularization was seen in the graft. Furthrmore, donor neurons were able to establish long-distance connections to the corpus callosum, and the scaffold showed little evidence of an immunological response.[149]

Each of these in vivo studies shows the promise of PGA, PLGA, and alginate materials in tissue engineering for the spinal cord and brain. Donor NSCs were able in some cases to aid in recovery from the injury and differentiate in vivo into different proportions of glial and neuronal cells. In addition, these observations were dependent on the chemical microenvironment created by the material, as well as its topological structure, since different results were seen with vs without the use of a scaffold. Furthermore, host neurons and glial cells were even able to incorporate into one of the scaffolds. Finally, results were generally better when both the cells and the scaffold were used, showing the combined promise of biomaterials and NSCs in tissue regeneration. Future work may explore the potential of bioactive materials to actively engage cellular signaling, as well as materials with controlled biodegradation properties.

4. Conclusions

Neural stem cells are very promising for the treatment of neurodegenerative disorders and injuries of the CNS. Engineered materials containing natural and/or synthetic components can support the expansion and potentially in the future induce the lineage-specific differentiation of NSCs in vitro, with a variety of applications ranging from cell replacement therapy to in vitro diagnostics and screens. Furthermore, highly modular systems that enable the independent variation of mechanical and multiple biochemical signals have strong potential for the application of reductionist biology approaches to understand fundamental mechanisms of stem cell behavioral regulation. However, a number of challenges remain in the design of materials that are nonimmunogenic, scalable, mechanically tunable, and bioactive in their presentation of key regulatory signals to cells. Synthetic materials have considerable promise for offering these capabilities, although challenges remain in the development of synthetic analogues of complex biochemical signals such as ECM proteins. If these challenges can be overcome, however, bioactive materials can be designed to present a microenvironment that can not only support cells in vitro but also protect them in the harsh environment of a diseased or injured region of the CNS and thereby greatly aid stem cell-based regenerative medicine.

5. List of Abbreviations

3D	three-dimensional
BMP	bone morphogenetic protein
BDNF	brain-derived neurotrophic factor
CNS	central nervous system
ECM	extracellular matrix
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
EVAL	poly(ethylene-co-vinyl alcohol)
FGF	fibroblast growth factor
GFP	green fluorescent protein
LAS	lysine-alanine sequential polymer
NPCs	neural precursor cells
NSCs	neural stem cells
PEG	poly(ethylene glycol)
PDMS	poly(dimethyl siloxane)
PGA	poly(glycolic acid)
PLGA	poly(lactic-co-glycolic acid)
PLLA	poly(L-lactic acid)
PS	polystyrene
PVA	polyvinyl alcohol
PVDF	poly(vinylidene fluoride)
RWV	rotating wall vessel
SAM	self-assembled monolayer
SVZ	subventricular zone
TEG	triethylene glycol
TGF	transforming growth factor

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7. References

(1) Gage, F. H. *Curr. Opin. Neurobiol.* **1998**, *8*, 671–676.
(2) Gage, F. H. *Science* **2000**, *287*, 1433–1438.
(3) Ma, W.; Liu, Q. Y.; Maric, D.; Sathanoori, R.; Chang, Y. H.; Barker, J. L. *J. Neurobiol.* **1998**, *35*, 277–286.
(4) Ma, W.; Maric, D.; Li, B. S.; Hu, Q.; Andreadis, J. D.; Grant, G. M.; Liu, Q. Y.; Shaffer, K. M.; Chang, Y. H.; Zhang, L.; Pancrazio, J. J.; Pant, H. C.; Stenger, D. A.; Barker, J. L. *Eur. J. Neurosci.* **2000**, *12*, 1227–1240.
(5) Maric, D.; Liu, Q. Y.; Grant, G. M.; Andreadis, J. D.; Hu, Q.; Chang, Y. H.; Barker, J. L.; Joseph, J.; Stenger, D. A.; Ma, W. *J. Neurosci. Res.* **2000**, *61*, 652–662.
(6) McKay, R. *Science* **1997**, *276*, 66–71.
(7) Ourednik, V.; Ourednik, J.; Flax, J. D.; Zawada, W. M.; Hutt, C.; Yang, C.; Park, K. I.; Kim, S. U.; Sidman, R. L.; Freed, C. R.; Snyder, E. Y. *Science* **2001**, *293*, 1820–1824.
(8) Shihabuddin, L. S.; Horner, P. J.; Ray, J.; Gage, F. H. *J. Neurosci.* **2000**, *20*, 8727–8735.
(9) Cameron, H. A.; Hazel, T. G.; McKay, R. D. *J. Neurobiol.* **1998**, *36*, 287–306.
(10) Jessell, T. M.; Melton, D. A. *Cell* **1992**, *68*, 257–270.
(11) Tsai, R. Y.; McKay, R. D. *J. Neurosci.* **2000**, *20*, 3725–3735.
(12) Young, T. H.; Hung, C. H. *Biomaterials* **2005**, *26*, 4291–4299.
(13) Carbonetto, S. T.; Gruver, M. M.; Turner, D. C. *Science* **1982**, *216*, 897–899.
(14) Ruoslahti, E. *Science* **1997**, *276*, 1345–1346.
(15) Streuli, C. *Curr. Opin. Cell Biol.* **1999**, *11*, 634–640.
(16) Alvarez-Buylla, A.; Lim, D. A. *Neuron* **2004**, *41*, 683–686.
(17) Bokel, C.; Brown, N. H. *Dev. Cell* **2002**, *3*, 311–321.
(18) Cattaneo, E.; McKay, R. *Nature* **1990**, *347*, 762–765.
(19) Davis, A. A.; Temple, S. *Nature* **1994**, *372*, 263–266.
(20) De Arcangelis, A.; Georges-Labouesse, E. *Trends Genet.* **2000**, *16*, 389–395.
(21) Giancotti, F. G.; Ruoslahti, E. *Science* **1999**, *285*, 1028–1032.
(22) Howe, A.; Aplin, A. E.; Alahari, S. K.; Juliano, R. L. *Curr. Opin. Cell Biol.* **1998**, *10*, 220–231.
(23) Mercier, F.; Kitasako, J. T.; Hatton, G. I. *J. Comp. Neurol.* **2002**, *451*, 170–188.
(24) Reynolds, B. A.; Weiss, S. *Science* **1992**, *255*, 1707–1710.
(25) Armstrong, R. J.; Watts, C.; Svendsen, C. N.; Dunnett, S. B.; Rosser, A. E. *Cell Transplant* **2000**, *9*, 55–64.
(26) Gage, F. H.; Coates, P. W.; Palmer, T. D.; Kuhn, H. G.; Fisher, L. J.; Suhonen, J. O.; Peterson, D. A.; Suhr, S. T.; Ray, J. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 11879–11883.
(27) Kim, S. U. *Neuropathology* **2004**, *24*, 159–171.
(28) Kim, S. U. *Brain Dev.* **2007**, *29*, 193–201.
(29) Lindvall, O.; Brundin, P.; Widner, H.; Rehncrona, S.; Gustavii, B.; Frackowiak, R.; Leenders, K. L.; Sawle, G.; Rothwell, J. C.; Marsden, C. D.; et al. *Science* **1990**, *247*, 574–547.
(30) Oka, S.; Honmou, O.; Akiyama, Y.; Sasaki, M.; Houkin, K.; Hashi, K.; Kocsis, J. D. *Brain Res.* **2004**, *1030*, 94–102.
(31) Pluchino, S.; Quattrini, A.; Brambilla, E.; Gritti, A.; Salani, G.; Dina, G.; Galli, R.; Del Carro, U.; Amadio, S.; Bergami, A.; Furlan, R.; Comi, G.; Vescovi, A. L.; Martino, G. *Nature* **2003**, *422*, 688–694.
(32) Studer, L.; Tabar, V.; McKay, R. D. *Nat. Neurosci.* **1998**, *1*, 290–295.
(33) Yandava, B. D.; Billinghurst, L. L.; Snyder, E. Y. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 7029–7034.
(34) Martinez-Serrano, A.; Bjorklund, A. *J. Neurosci.* **1996**, *16*, 4604–4616.
(35) Brustle, O.; Spiro, A. C.; Karram, K.; Choudhary, K.; Okabe, S.; McKay, R. D. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 14809–14814.
(36) Fricker, R. A.; Carpenter, M. K.; Winkler, C.; Greco, C.; Gates, M. A.; Bjorklund, A. *J. Neurosci.* **1999**, *19*, 5990–6005.
(37) Suhonen, J. O.; Peterson, D. A.; Ray, J.; Gage, F. H. *Nature* **1996**, *383*, 624–627.
(38) Akerud, P.; Canals, J. M.; Snyder, E. Y.; Arenas, E. *J. Neurosci.* **2001**, *21*, 8108–8118.
(39) Liu, S.; Qu, Y.; Stewart, T. J.; Howard, M. J.; Chakrabortty, S.; Holekamp, T. F.; McDonald, J. W. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6126–6131.
(40) Snyder, E. Y.; Taylor, R. M.; Wolfe, J. H. *Nature* **1995**, *374*, 367–370.
(41) Snyder, E. Y.; Yoon, C.; Flax, J. D.; Macklis, J. D. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 11663–11668.
(42) Guillaume, D. J.; Johnson, M. A.; Li, X. J.; Zhang, S. C. *J. Neurosci. Res.* **2006**, *84*, 1165–1176.
(43) Li, X.-J.; Du, Z.-W.; Zarnowska, E.; Pankratz, M.; Hansen, L.; Pearce, R.; Zhang, S.-C. *Nat. Biotechnol.* **2005**, *23*, 215–221.
(44) Mueller, D.; Shamblott, M. J.; Fox, H. E.; Gearhart, J. D.; Martin, L. J. *J. Neurosci. Res.* **2005**, *82*, 592–608.
(45) Sanchez-Pernaute, R.; Studer, L.; Ferrari, D.; Perrier, A.; Lee, H.; Vinuela, A.; Isacson, O. *Stem Cells* **2005**, *23*, 914–922.
(46) Zeng, X.; Cai, J.; Chen, J.; Luo, Y.; You, Z. B.; Fotter, E.; Wang, Y.; Harvey, B.; Miura, T.; Backman, C.; Chen, G. J.; Rao, M. S.; Freed, W. J. *Stem Cells* **2004**, *22*, 925–940.
(47) Zietlow, R.; Pekarik, V.; Armstrong, R. J.; Tyers, P.; Dunnett, S. B.; Rosser, A. E. *J. Anat.* **2005**, *207*, 227–240.
(48) Martin, M. J.; Muotri, A.; Gage, F.; Varki, A. *Nat. Med.* **2005**, *11*, 228–232.
(49) Xu, C.; Inokuma, M.; Denham, J.; Golds, K.; Kundu, P.; Gold, J.; Carptenter, M. *Nat. Biotechnol.* **2001**, *19*, 971–974.
(50) Yao, S.; Chen, S.; Clark, J.; Hao, E.; Beattie, G.; Hayek, A.; Ding, S. *Proc. Natl. Acad. Sci.* **2006**, *103*, 6907–6912.
(51) Xu, C.; Rosler, E.; Jiang, J.; Lebkowski, J.; Gold, J.; O'Sullivan, C.; Delavan-Boorsma, K.; Mok, M.; Bronstein, A.; Carpenter, M. *Stem Cells* **2005**, *23*, 315–323.
(52) Li, Y.; Powell, S.; Brunette, E.; Lebkowski, J.; Mandalam, R. *Biotechnol. Bioeng.* **2005**, *91*, 688–698.
(53) Liu, Y.; Song, Z.; Zhao, Y.; Qin, H.; Cai, J.; Zhang, H.; Yu, T.; Jiang, S.; Wang, G.; Ding, M.; Deng, H. *Biochem. Biophys. Res. Commun.* **2006**, *346*, 131–139.
(54) Genbacev, O.; Krtolica, A.; Zdravkovic, T.; Brunette, E.; Powell, S.; Nath, A.; Caceres, E.; McMaster, M.; McDonagh, S.; Li, Y.; Mandalam, R.; Lebkowski, J.; Fisher, S. J. *Fertil. Steril.* **2005**, *83*, 1517–1529.
(55) Ruoslahti, E.; Pierschbacher, M. D. *Science* **1987**, *238*, 491–497.
(56) Schense, J. C.; Bloch, J.; Aebischer, P.; Hubbell, J. A. *Nat. Biotechnol.* **2000**, *18*, 415–419.
(57) Lutolf, M. P.; Hubbell, J. A. *Nat. Biotechnol.* **2005**, *23*, 47–55.
(58) Healy, K. E.; Rezania, A.; Stile, R. A. *Ann. N. Y. Acad. Sci.* **1999**, *875*, 24–35.
(59) Engler, A. J.; Sen, S.; Sweeney, H. L.; Discher, D. E. *Cell* **2006**, *126*, 677–689.
(60) Georges, P. C.; Miller, W. J.; Meaney, D. F.; Sawyer, E. S.; Janmey, P. A. *Biophys. J.* **2006**, *90*, 3012–3018.
(61) Janmey, P. A.; McCulloch, C. A. *Annu. Rev. Biomed. Eng.* **2007**, *9*, 1–34.
(62) Stewart, R.; Przyborski, S. *Bioessays* **2002**, *24*, 708–713.
(63) Friess, W. *Eur. J. Pharm. Biopharm.* **1998**, *45*, 113–136.
(64) Orive, G.; Ponce, S.; Hernandez, R. M.; Gascon, A. R.; Igartua, M.; Pedraz, J. L. *Biomaterials* **2002**, *23*, 3825–3831.
(65) Shapiro, L.; Cohen, S. *Biomaterials* **1997**, *18*, 583–590.
(66) Suzuki, K.; Suzuki, Y.; Ohnishi, K.; Endo, K.; Tanihara, M.; Nishimura, Y. *Neuroreport* **1999**, *10*, 2891–2894.
(67) Kerever, A.; Schnack, J.; Vellinga, D.; Ichikawa, N.; Moon, C.; Arikawa-Hirasawa, E.; Efird, J. T.; Mercier, F. *Stem Cells* **2007**, *25*, 2146–2157.
(68) Venstrom, K. A.; Reichardt, L. F. *FASEB J.* **1993**, *7*, 996–1003.
(69) Wright, J. W.; Harding, J. W. *Prog. Neurobiol.* **2004**, *72*, 263–293.
(70) Lin, H. J.; O'Shaughnessy, T. J.; Kelly, J.; Ma, W. *Brain Res. Dev. Brain Res.* **2004**, *153*, 163–173.
(71) Ma, W.; Fitzgerald, W.; Liu, Q. Y.; O'Shaughnessy, T. J.; Maric, D.; Lin, H. J.; Alkon, D. L.; Barker, J. L. *Exp. Neurol.* **2004**, *190*, 276–288.
(72) O'Connor, S. M.; Andreadis, J. D.; Shaffer, K. M.; Ma, W.; Pancrazio, J. J.; Stenger, D. A. *Biosens. Bioelectron.* **2000**, *14*, 871–881.
(73) O'Connor, S. M.; Stenger, D. A.; Shaffer, K. M.; Maric, D.; Barker, J. L.; Ma, W. *J. Neurosci. Methods* **2000**, *102*, 187–195.
(74) Low, H. P.; Savarese, T. M.; Schwartz, W. J. *In Vitro Cell Dev. Biol. Anim.* **2001**, *37*, 141–147.
(75) Sanford, G. L.; Ellerson, D.; Melhado-Gardner, C.; Sroufe, A. E.; Harris-Hooker, S. *In Vitro Cell Dev. Biol. Anim.* **2002**, *38*, 493–504.
(76) Whittemore, S. R.; Morassutti, D. J.; Walters, W. M.; Liu, R. H.; Magnuson, D. S. *Exp. Cell Res.* **1999**, *252*, 75–95.
(77) Gritti, A.; Parati, E. A.; Cova, L.; Frolichsthal, P.; Galli, R.; Wanke, E.; Faravelli, L.; Morassutti, D. J.; Roisen, F.; Nickel, D. D.; Vescovi, A. L. *J. Neurosci.* **1996**, *16*, 1091–1100.
(78) Kilpatrick, T. J.; Bartlett, P. F. *J. Neurosci.* **1995**, *15*, 3653–3661.
(79) Harmer, N. J. *Biochem. Soc. Trans.* **2006**, *34*, 442–445.
(80) Flanagan, L. A.; Rebaza, L. M.; Derzic, S.; Schwartz, P. H.; Monuki, E. S. *J. Neurosci. Res.* **2006**, *83*, 845–856.
(81) Soen, Y.; Mori, A.; Palmer, T. D.; Brown, P. O. *Mol. Syst. Biol.* **2006**, *2*, 37.
(82) Nakajima, M.; Ishimuro, T.; Kato, K.; Ko, I. K.; Hirata, I.; Arima, Y.; Iwata, H. *Biomaterials* **2007**, *28*, 1048–1060.
(83) Anderson, J. P.; Cappello, J.; Martin, D. C. *Biopolymers* **1994**, *34*, 1049–1058.
(84) Ashton, R. S.; Peltier, J.; Fasano, C. A.; O'Neill, A.; Leonard, J.; Temple, S.; Schaffer, D. V.; Kane, R. S. *Stem Cells* **2007**, *25*, 2928–2935.
(85) Recknor, J. B.; Sakaguchi, D. S.; Mallapragada, S. K. *Biomaterials* **2006**, *27*, 4098–4108.

(86) Lim, D. A.; Alvarez-Buylla, A. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 7526–7531.
(87) Song, H.; Stevens, C. F.; Gage, F. H. *Nature* **2002**, *417*, 39–44.
(88) Li, X.; Liu, T.; Song, K.; Yao, L.; Ge, D.; Bao, C.; Ma, X.; Cui, Z. *Biotechnol. Prog.* **2006**, *22*, 1683–1689.
(89) Prang, P.; Muller, R.; Eljaouhari, A.; Heckmann, K.; Kunz, W.; Weber, T.; Faber, C.; Vroemen, M.; Bogdahn, U.; Weidner, N. *Biomaterials* **2006**, *27*, 3560–3569.
(90) Ashton, R. S.; Banerjee, A.; Punyani, S.; Schaffer, D. V.; Kane, R. S. *Biomaterials* **2007**, *28*, 5518–5525.
(91) Soria, J. M.; Martinez Ramos, C.; Salmeron Sanchez, M.; Benavent, V.; Campillo Fernandez, A.; Gomez Ribelles, J. L.; Garcia Verdugo, J. M.; Pradas, M. M.; Barcia, J. A. *J. Biomed. Mater. Res. A* **2006**, *79*, 495–502.
(92) Bini, T. B.; Gao, S.; Wang, S.; Ramakrishna, S. *J. Mater. Sci. Mater. Med.* **2005**, *16*, 367–375.
(93) Matsuda, A.; Kobayashi, H.; Itoh, S.; Kataoka, K.; Tanaka, J. *Biomaterials* **2005**, *26*, 2273–2279.
(94) Garcia, A. J.; Vega, M. D.; Boettiger, D. *Mol. Biol. Cell* **1999**, *10*, 785–798.
(95) Keselowsky, B. G.; Collard, D. M.; Garcia, A. J. *Biomaterials* **2004**, *25*, 5947–5954.
(96) Keselowsky, B. G.; Collard, D. M.; Garcia, A. J. *J. Biomed. Mater. Res. A* **2003**, *66*, 247–259.
(97) Sherratt, M. J.; Bax, D. V.; Chaudhry, S. S.; Hodson, N.; Lu, J. R.; Saravanapavan, P.; Kielty, C. M. *Biomaterials* **2005**, *26*, 7192–7206.
(98) Tate, M. C.; Garcia, A. J.; Keselowsky, B. G.; Schumm, M. A.; Archer, D. R.; LaPlaca, M. C. *Mol. Cell. Neurosci.* **2004**, *27*, 22–31.
(99) Mahoney, M. J.; Anseth, K. S. *J. Biomed. Mater. Res. A* **2007**, *81*, 269–278.
(100) Nakaji-Hirabayashi, T.; Kato, K.; Arima, Y.; Iwata, H. *Biomaterials* **2007**, *28*, 3517–3529.
(101) Kato, K.; Sato, H.; Iwata, H. *Langmuir* **2005**, *21*, 7071–7075.
(102) Ko, I. K.; Kato, K.; Iwata, H. *Biomaterials* **2005**, *26*, 4882–4891.
(103) Silva, G. A.; Czeisler, C.; Niece, K. L.; Beniash, E.; Harrington, D. A.; Kessler, J. A.; Stupp, S. I. *Science* **2004**, *303*, 1352–1355.
(104) Holmes, T. C.; de Lacalle, S.; Su, X.; Liu, G.; Rich, A.; Zhang, S. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6728–6733.
(105) Ranieri, J. P.; Bellamkonda, R.; Bekos, E. J.; Vargo, T. G.; Gardella, J. A., Jr.; Aebischer, P. *J. Biomed. Mater. Res.* **1995**, *29*, 779–785.
(106) Fischer, S. E.; Liu, X.; Mao, H. Q.; Harden, J. L. *Biomaterials* **2007**, *28*, 3325–3337.
(107) Lombardi, A.; Bryson, J. W.; DeGrado, W. F. *Biopolymers* **1996**, *40*, 495–504.
(108) Mi, L.; Fischer, S.; Chung, B.; Sundelacruz, S.; Harden, J. L. *Biomacromolecules* **2006**, *7*, 38–47.
(109) Petka, W. A.; Harden, J. L.; McGrath, K. P.; Wirtz, D.; Tirrell, D. A. *Science* **1998**, *281*, 389–392.
(110) Hartgerink, J. D.; Beniash, E.; Stupp, S. I. *Science* **2001**, *294*, 1684–1688.
(111) Hartgerink, J. D.; Beniash, E.; Stupp, S. I. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5133–5138.
(112) Chen, Z. J.; Ughrin, Y.; Levine, J. M. *Mol. Cell. Neurosci.* **2002**, *20*, 125–139.
(113) Costa, S.; Planchenault, T.; Charriere-Bertrand, C.; Mouchel, Y.; Fages, C.; Juliano, S.; Lefrancois, T.; Barlovatz-Meimon, G.; Tardy, M. *Glia* **2002**, *37*, 105–113.
(114) Rabchevsky, A. G.; Smith, G. M. *Arch. Neurol.* **2001**, *58*, 721–726.
(115) Gelain, F.; Bottai, D.; Vescovi, A.; Zhang, S. *PLoS ONE* **2006**, *1*, e119.
(116) Zhang, S.; Holmes, T.; Lockshin, C.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 3334–3338.
(117) Zhang, S.; Holmes, T. C.; DiPersio, C. M.; Hynes, R. O.; Su, X.; Rich, A. *Biomaterials* **1995**, *16*, 1385–1393.
(118) Nowakowski, G. S.; Dooner, M. S.; Valinski, H. M.; Mihaliak, A. M.; Quesenberry, P. J.; Becker, P. S. *Stem Cells* **2004**, *22*, 1030–1038.
(119) Semino, C. E.; Kasahara, J.; Hayashi, Y.; Zhang, S. *Tissue Eng.* **2004**, *10*, 643–655.
(120) Yang, F.; Murugan, R.; Wang, S.; Ramakrishna, S. *Biomaterials* **2005**, *26*, 2603–2610.
(121) Anderson, D. G.; Putnam, D.; Lavik, E. B.; Mahmood, T. A.; Langer, R. *Biomaterials* **2005**, *26*, 4892–4897.
(122) Wang, J. H.; Hung, C. H.; Young, T. H. *Biomaterials* **2006**, *27*, 3441–3450.
(123) Brewer, G. J.; Deshmane, S.; Ponnusamy, E. *J. Neurosci. Methods* **1998**, *85*, 13–20.
(124) Engel, J.; Kurtz, J.; Katchalski, E.; Berger, A. *J. Mol. Biol.* **1966**, *17*, 255–272.
(125) Young, T. H.; Huang, J. H.; Hung, S. H.; Hsu, J. P. *J. Biomed. Mater. Res.* **2000**, *52*, 748–753.
(126) Kilpatrick, T. J.; Bartlett, P. F. *Neuron* **1993**, *10*, 255–265.
(127) Hulspas, R.; Tiarks, C.; Reilly, J.; Hsieh, C. C.; Recht, L.; Quesenberry, P. J. *Exp. Neurol.* **1997**, *148*, 147–156.
(128) Xu, C.; Yang, F.; Wang, S.; Ramakrishna, S. *J. Biomed. Mater. Res. A* **2004**, *71*, 154–161.
(129) Yang, F.; Murugan, R.; Ramakrishna, S.; Wang, X.; Ma, Y. X.; Wang, S. *Biomaterials* **2004**, *25*, 1891–1900.
(130) Young, T. H.; Chuang, W. Y.; Hsieh, M. Y.; Chen, L. W.; Hsu, J. P. *Biomaterials* **2002**, *23*, 3495–3501.
(131) Young, T. H.; Chuang, W. Y.; Yao, N. K.; Chen, L. W. *J. Biomed. Mater. Res.* **1998**, *40*, 385–391.
(132) Young, T. H.; Hu, W. W. *Biomaterials* **2003**, *24*, 1477–1486.
(133) Young, T. H.; Lin, C. W.; Cheng, L. P.; Hsieh, C. C. *Biomaterials* **2001**, *22*, 1771–1777.
(134) Young, T. H.; Yao, C. H.; Sun, J. S.; Lai, C. P.; Chen, L. W. *Biomaterials* **1998**, *19*, 717–724.
(135) Hung, C. H.; Lin, Y. L.; Young, T. H. *Biomaterials* **2006**, *27*, 4461–4469.
(136) Aebischer, P.; Valentini, R. F.; Dario, P.; Domenici, C.; Galletti, P. M. *Brain Res.* **1987**, *436*, 165–168.
(137) Guenard, V.; Valentini, R. F.; Aebischer, P. *Biomaterials* **1991**, *12*, 259–263.
(138) Okoshi, T.; Chen, H.; Soldani, G.; Galletti, P. M.; Goddard, M. *Asaio J.* **1992**, *38*, M201-6.
(139) Saha, K.; Irwin, E. F.; Kozhukh, J.; Schaffer, D. V.; Healy, K. E. *J. Biomed. Mater. Res. A* **2007**, *81*, 240–249.
(140) Bearinger, J. P.; Castner, D. G.; Golledge, S. L.; Rezania, A.; Hubchak, S.; Healy, K. E. *Langmuir* **1997**, *13*, 5175–5183.
(141) Barber, T. A.; Gamble, L. J.; Castner, D. G.; Healy, K. E. *J. Orthop. Res.* **2006**, *24*, 1366–1376.
(142) Harbers, G. M.; Gamble, L. J.; Irwin, E. F.; Castner, D. G.; Healy, K. E. *Langmuir* **2005**, *21*, 8374–8384.
(143) Ho, J. E.; Chung, E. H.; Wall, S.; Schaffer, D. V.; Healy, K. E. *J. Biomed. Mater. Res. A* **2007**, *83*, 1200–1208.
(144) Geraerts, M.; Krylyshkina, O.; Debyser, Z.; Baekelandt, V. *Stem Cells* **2007**, *25*, 263–270.
(145) Yasuhara, T.; Matsukawa, N.; Hara, K.; Yu, G.; Xu, L.; Maki, M.; Kim, S. U.; Borlongan, C. V. *J. Neurosci.* **2006**, *26*, 12497–12511.
(146) Ziv, Y.; Avidan, H.; Pluchino, S.; Martino, G.; Schwartz, M. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 13174–13179.
(147) Hill, E.; Boontheekul, T.; David, M. *Proc. Natl. Acad. Sci.* **2006**, *103*, 2494–2499.
(148) Lee, J. P.; Jeyakumar, M.; Gonzalez, R.; Takahashi, H.; Lee, P. J.; Baek, R. C.; Clark, D.; Rose, H.; Fu, G.; Clarke, J.; McKercher, S.; Meerloo, J.; Muller, F. J.; Park, K. I.; Butters, T. D.; Dwek, R. A.; Schwartz, P.; Tong, G.; Wenger, D.; Lipton, S. A.; Seyfried, T. N.; Platt, F. M.; Snyder, E. Y. *Nat. Med.* **2007**, *13*, 439–447.
(149) Park, K. I.; Teng, Y. D.; Snyder, E. Y. *Nat. Biotechnol.* **2002**, *20*, 1111–1117.
(150) Wu, S.; Suzuki, Y.; Kitada, M.; Kitaura, M.; Kataoka, K.; Takahashi, J.; Ide, C.; Nishimura, Y. *Neurosci. Lett.* **2001**, *312*, 173–176.
(151) Sufan, W.; Suzuki, Y.; Tanihara, M.; Ohnishi, K.; Suzuki, K.; Endo, K.; Nishimura, Y. *J. Neurotrauma* **2001**, *18*, 329–338.
(152) Vacanti, M. P.; Leonard, J. L.; Dore, B.; Bonassar, L. J.; Cao, Y.; Stachelek, S. J.; Vacanti, J. P.; O'Connell, F.; Yu, C. S.; Farwell, A. P.; Vacanti, C. A. *Transplant Proc.* **2001**, *33*, 592–598.
(153) Teng, Y. D.; Lavik, E. B.; Qu, X.; Park, K. I.; Ourednik, J.; Zurakowski, D.; Langer, R.; Snyder, E. Y. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 3024–3029.
(154) Kim, B. S.; Mooney, D. J. *Trends Biotechnol.* **1998**, *16*, 224–230.

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