Extracellular matrices, artificial neural scaffolds and the promise of neural regeneration

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Abstract

Over last 20 years, extracellular matrices have been shown to be useful in promoting tissue regeneration. Recently, they have been used and have had success in achieving neurogenesis. Recent developments in extracellular matrix design have allowed their successful in vivo incorporation to engender an environment favorable for neural regeneration in animal models. Promising treatments under investigation include manipulation of the intrinsic extracellular matrix and incorporation of engineered nanometer-sized scaffolds through which the inhibition of molecules serving as barriers to neuroregeneration and delivery of neurotrophic factors and/or cells for successful tissue regeneration can be achieved. Further understanding of the changes incurred within the extracellular matrix following central nervous system injury will undoubtedly help design a clinically efficacious extracellular matrix scaffold that can mitigate or reverse neural degeneration in the clinical setting.

Key Words: extracellular matrix; neurogenesis; scaffolds; neural stem cells

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Introduction

For many years, management of neurological injury secondary to trauma or stroke has focused on mediating the cellular changes that cause secondary damage (Greve et al., 2009). Advances in the understanding of the role of extracellular matrix (ECM) in influencing neurogenesis, however, have presented novel strategies for tissue regeneration (Hallbergson et al., 2003). In this review, we discuss the functions of the ECM and summarize the basic science advances and several novel treatments under investigation that highlight the potential for intrinsically altering the ECM and the use of artificial ECM scaffolds to achieve neural regeneration.

The repair of a damaged axon involves a complex series of cellular changes to coordinate the formation of a growth cone which can successfully follow a sea of inhibitory and permissive signals to reunite with its prior synapses and properly regenerate (Szpara et al., 2007). In the peripheral nervous system (PNS), these changes are called Wallerian degeneration. Here, denervated Schwann cells differentiate and proliferate, producing neurotropic molecules, cell receptors, and basement membrane elements which allow the growth cone to reach its original target. In the central nervous system (CNS), in which oligodendrocytes myelinate multiple neurons, injury causes more widespread demyelination. Injury to the CNS causes a common pathway of destruction through secondary injuries like hemorrhage, edema, ischemia, and inflammation (Fitch et al., 1997). In spinal cord injuries, these secondary injuries lead to necrosis and apoptosis in 1–2 segments above the original site of injury, with reactive astrocytes forming a glial scar (Faulkner et al., 2004). Wallerian degeneration like that seen in the PNS is furthermore impeded by the inhibiting properties of slowly cleared myelin degradation products (McKerracher et al., 1994) and the absence of an organized basal lamina. The glial scar, itself, contains inhibitory molecules (Fawcett et al., 1997) which, at first, limit areas of damage by reestablishing the blood-brain barrier and containing inflammation and released neurotransmitters (Faulkner et al., 2004); however, overtime, the ECM deposited within the glial scar contains inhibitory molecules like chondroitin sulfate proteoglycans (CSPGs) which prevent further neurogenesis (McKeon et al., 1991).

The ECM

In recent years, the role of the ECM in regulating the survival, differentiation, growth, and migration of cells in the nervous system and its impact on neurogenesis have been increasingly characterized. The ECM, which constitutes 10–20% of the brain's volume, is composed of a network of proteins and glycans which are produced intracellularly and get secreted into the extracellular space (Bignami et al., 1993). In addition to its important structural, anchoring, and organizing functions, the ECM also contains signals which regulate growth, differentiation, synapse remodeling, and migration (Bandlow et al., 2000; Dityatev et al., 2003). The network is organized roughly into three compartments (Figure 1): the...
basement membrane (basal lamina) that surrounds cerebral vessels, the perineuronal nets (PNNs) surrounding cell bodies and dendrites, and a diffusely distributed interstitial matrix lying between CNS parenchyma (Lau et al., 2013). The basement membrane is composed of collagen, fibronectin, perlecain, dystroglycan and laminin-nidogen (entactin) complexes. It surrounds the pial surface of the CNS and separates the endothelial cells from parenchyma, thus forming the blood-brain barrier. During cerebral ischemia, increased expression of matrix metalloproteinases 9 and 2 results in the breakdown of the basement membrane, which may facilitate hemorrhage, edema, and secondary injury (Wang et al., 2007). PNNs are primarily composed of a dense matrix of hyaluronan (HA), chondroitin sulfate proteoglycans (CSPGs), tenascin R and link proteins, and serve as crucial regulators of synaptic plasticity (Kwok et al., 2011). The negatively charged glycosaminoglycans (GAG) regulate the diffusion of important cations, such as sodium, potassium, and calcium, which supports rapid neuronal firing (Hartig et al., 1999).

**ECM modifications following injury**

CNS injury causes the proliferation of astrocytes, fibroblasts, and oligodendrocyte precursors which form a glial scar (Hatten et al., 1991; Chen et al., 2002). Within this glial scar, upregulated proteoglycans like CSPGs and changes in sulfation patterns within the ECM itself result in the creation of a barrier to regeneration (Massey et al., 2008). Multiple studies using the bacterial enzyme ChaseABC to digest chondroitin sulfate chains in CSPGs have demonstrated reversal of these inhibitory functions (McKeon et al., 1995). Promising in vitro work led to successful in vivo injection of ChaseABC into rats with spinal cord contusion injuries, leading to successful CSPG degradation (Lemons et al., 1999). Multiple other models have since demonstrated post-injury removal of chondroitin sulfates, promoting axon regeneration (Moon et al., 2001), and even functional recovery in rats with lesioned dorsal columns (Bradbury et al., 2002) and cats with spinal cord hemisection (Tester et al., 2008). Alternative strategies besides injection to deliver ChaseABC to modify the PNN in injured areas have included transgenic astrocytes with a GFAP promoter (Cafferty et al., 2008), neutralization with antibodies (Tan et al., 2006), and siRNA knocking down CS GAG elongation enzymes (Laabs et al., 2007).

**Artificial scaffolds**

Besides manipulating the intrinsic extracellular matrix to engender an environment favoring neural regeneration, an alternative strategy involves the use of artificially created, nanometer-sized scaffolds to overcome the body's natural barriers to repair. The use of a tissue-engineered scaffold offers a way to the poor regenerative capacity of the CNS to reconstruct formed cavities and reconnect neuronal processes. Thus, the artificial scaffold functions to enhance the communication between cells, allowing for improvement in proliferation, migration, and differentiation (Figure 2). Studies have demonstrated efficacy of bare scaffolds within the CNS (Huang et al., 2012), but regeneration and functional recovery in lesioned rat
models have been enhanced with the addition of other substrates such as vascular endothelial growth factor (Zhang et al., 2007) or hyaluronic acid with laminin (Hou et al., 2005).

Increasingly, stem cells have been incorporated into these engineered scaffolds to support three-dimensional cell organization. Neural stem cells (NSCs) are the multipotent, self-renewing progenitors of neurons, astrocytes, and oligodendrocytes. Three distinct populations exist in the subventricular zone, external germinal layer of the cerebellum, and subgranular layer of the dentate gyrus (Doetsch et al., 1999). Cells can be harvested directly from these regions in adult or embryonic tissues, or by inducing embryonic stem cell (ESCs) differentiation, which may have less fate restrictions but greater tumorigenic potential (Nishikawa et al., 2007). In addition, cells taken from other areas, such as marrow stromal cells (MSCs), olfactory ensheathing cells, or Schwann cells, can give rise to cells for autotransplantation in a minimally-invasive and easily accessible process (Hayase et al., 2009). Transplantation can aid neuroregeneration not only by differentiating to replace the damaged or degenerative cell types (Zhu et al., 2009), but also by secreting various neurotrophic factors like nerve growth factor and brain-derived neurotrophic factor (BDNF) (Lu et al., 2003), and by inhibiting T cell activation, thus preventing further injury (Bacigaluppi et al., 2009). Schwann cells promote CNS regeneration by naturally expressing numerous surface adhesion molecules and growth factors, and by producing the ECM components laminin and fibronectin (Volpato et al., 2013).

The use of biomaterials and polymers has been shown to be an essential strategy to maximize cell transplantation efficacy. Indeed, the success of cell transplantation and eventual neurogenesis is a complex process that relies upon: a) the delivery of the stem cells into a framework where cells can interact, b) the appropriate signaling molecules within the microenvironment, and c) an adequate blood supply. The ideal cell scaffold is physicochemically similar to the surrounding tissues, non-toxic, poorly immunogenic, biodegradable, durable, cheap, conformable to various structures, and highly porous to allow for a high density of cell seeding (Madigan et al., 2009). These scaffolds appear in various forms, including hydrogels, sponges, membranes, and channel containing tubules, and can be from naturally occurring or synthetic components.

Natural scaffolds made of proteins and carbohydrates are generally highly biocompatible and easily available. As one of the major components of ECM, collagen has been well studied. In combination with neurotropic factors like BDNF and neurotrophin-3 (NT-3), axonal regeneration and partial functional recovery after dorsal spinal cord transection have been noted (East et al., 2010; Han et al., 2010). Addition of NSCs also demonstrated improved remyelination and recovery in rat lesioned models (Hatami et al., 2009), and combination delivery with ChaseABC also showed improved bladder function (Fouda et al., 2009). Fibrin, a natural component of blood clots, can also be used as an injected or biodegradable scaffold to promote migration of neural support cells and promote implanted NSC survival (Johnson et al., 2010). Other naturally occurring scaffolds include hyaluronic acid, which has lymphocyte and astrocyte inhibition properties and has been shown to lead to diminished glial scar formation (Wei et al., 2010), or alginate, agarose, and chitin sponges or gels, which have proved a versatile delivery vehicle for various cells and growth factors (Kataoka et al., 2004; Stokols et al., 2006; Bozkurt et al., 2010; Barminko et al., 2011).

Artificial scaffolds, designed to mimic endogenous ECM, formed from type I collagen and glycosaminoglycans (CGs), have already been successfully used to facilitate regeneration of skin, bone, and peripheral nerves (Yannas et al., 2010). Synthetic scaffolds are an attractive alternative to animal-derived biomaterials due to their minimal risk of pathogen transmission (Kyle et al., 2009), similar structure to naturally occurring ECM (Zhang et al., 2005), ease of liquid delivery to in vivo targets, and lack of immune response (Rudra et al., 2010).

Polymeric scaffolds designed for the CNS rely upon the self-assembling properties of engineered peptides, whereas a liquid monomer can be injected to spontaneously form highly ordered architectures (Bonzani et al., 2006). The list of synthetic compounds is rapidly expanding, and includes compounds like poly-E-caprolactone, poly-lactic acid, poly-lactic-co-glycolic acid, poly-B-hydroxybutirate, polyethylene glycol, and poly (2-hydroxyethyl methacrylate) (Kim et al., 2014). These can be utilized as sponges, sheets, channels, films, or hydrogels. Hydrogels, from either natural or synthetic compounds are easily implanted by simple injection and conform easily to surrounding tissue (Phillips et al., 2004). Guidance channels, derived from synthetic biomaterials, may prove superior at axonal regeneration, but must be surgically implanted, given their structural stiffness (Wong et al., 2008). In addition to NSCs, MSCs, Schwann cells, and epithelial stem cells are transplanted either alone or together for improved efficacy (Oh et al., 2011). Likewise, growth factors most commonly used in combination include NT-3, FGF-2, GDNF, and BDNF (Zurita et al., 2010).
Conclusion

Given the heterogenous nature of injury to the CNS through ischemia, trauma, or degeneration to diverse anatomic locations and cell populations, it is not surprising that therapeutic strategies have also become increasingly complex. Manipulating the extracellular matrix allows for the delivery of targeted scaffolds containing tailored combinations of cells and neurotrophic factors, many of which have demonstrated marked efficacy in animal models. Although the application of these findings has yet to significantly alter the way clinicians view or treat these conditions, there is promise for significant therapeutic advances in our lifetime.

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