

Novel Tools and Methods

Developing Extracellular Matrix Technology to Treat Retinal or Optic Nerve Injury

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Abstract

Adult mammalian CNS neurons often degenerate after injury, leading to lost neurologic functions. In the visual system, retinal or optic nerve injury often leads to retinal ganglion cell axon degeneration and irreversible vision loss. CNS axon degeneration is increasingly linked to the innate immune response to injury, which leads to tissue-destructive inflammation and scarring. Extracellular matrix (ECM) technology can reduce inflammation, while increasing functional tissue remodeling, over scarring, in various tissues and organs, including the peripheral nervous system. However, applying ECM technology to CNS injuries has been limited and virtually unstudied in the visual system. Here we discuss advances in deriving fetal CNS-specific ECMs, like fetal porcine brain, retina, and optic nerve, and fetal non-CNS-specific ECMs, like fetal urinary bladder, and the potential for using tissue-specific ECMs to treat retinal or optic nerve injuries in two platforms. The first platform is an ECM hydrogel that can be administered as a retrobulbar, periocular, or even intraocular injection. The second platform is an ECM hydrogel and polymer “biohybrid” sheet that can be readily shaped and wrapped around a nerve. Both platforms can be tuned mechanically and biochemically to deliver factors like neurotrophins, immunotherapeutics, or stem cells. Since clinical CNS therapies often use general anti-inflammatory agents, which can reduce tissue-destructive inflammation but also suppress tissue-reparative immune system functions, tissue-specific, ECM-based devices may fill an important need by providing naturally derived, biocompatible, and highly translatable platforms that can modulate the innate immune response to promote a positive functional outcome.

Key words: axon regeneration; ECM; immunotherapy; regenerative medicine; retinal ganglion cell

Significance Statement

Failed regeneration in CNS neurons is increasingly linked to the innate response of the immune system to injury, which leads to tissue destructive inflammation and scarring. Extracellular matrix (ECM) technology has been widely successful clinically in modulating the innate response of the immune system to reduce inflammation and to increase positive tissue remodeling, over scarring. ECM technology is now being developed to treat CNS injuries. Here we discuss recent advances in developing ECM technology in two platforms, an injectable ECM hydrogel and an ECM hydrogel and polymer “biohybrid” sheet. Unlike traditional immunosuppressive treatments that also suppress beneficial immune system functions, ECM-based devices offer a natural biocompatible platform for modulating the innate immune response to promote functional CNS tissue repair.

Introduction

Approximately 2.5 million cases of ocular trauma are reported annually in the United States, with about 50,000 of these cases resulting in permanent vision loss at an estimated lifetime cost of approximately \$900,000 per person according to the National Federation of the Blind (2013). Additionally, ~80 million people in the United States experience eye-blinding diseases. Worldwide, ~285 million people are visually impaired, and this number is predicted to increase with increasing longevity (Pascolini and Mariotti, 2012). However, we lack a clinically relevant regenerative medicine approach to promoting constructive tissue remodeling in the neural tissues of the eye, the retina, and the optic nerve. This review discusses developing extracellular matrix (ECM) hydrogel technology to promote constructive tissue remodeling, over scarring, after trauma or disease by modulating the innate immune response to injury. Tissue-specific ECM hydrogels may also provide a more biologically relevant delivery platform for existing retinal and optic nerve repair technologies, like delivering neurotrophic factors, stem or progenitor cells, or antioxidants, among others. Though this review focuses primarily on the retinal ganglion cells (RGCs), microglia, and macrophages, the potential benefits of ECM technology are widely applicable to other neural and glial populations throughout the CNS.

In adult mammalian CNS neurons, failed axon regeneration remains a persistent problem due to the variety of factors prohibiting axon regeneration. In the visual system, injury to RGC axons often leads to progressive RGC axon degeneration, and ultimately to RGC death and permanent vision loss. The inability of CNS neurons to regenerate injured axons is due to multiple intrinsic and injury-induced factors that suppress axon regeneration, including poor intrinsic axon growth ability (Goldberg et al., 2002), altered organelle dynamics (Lathrop and Steketeer, 2013), lost neurotrophic support, (Mansour-

Robaey et al., 1994), glial expressed inhibitory molecules like Nogo-A, myelin-associated glycoprotein, and oligodendrocyte myelin glycoprotein (McKeon et al., 1999; Tang et al., 2001), and the innate immune response (Horn et al., 2008; Fig. 1), among others.

After injury, RGCs can regenerate axons over long distances, if provided a suitable substrate like a peripheral nerve graft, to reinnervate visual centers in the rodent brain (Vidal-Sanz et al., 1987). In rodent models, transected RGC axons initially display transitory axonal sprouting, indicating that some intrinsic capacity for regeneration exists, and then ~90% undergo apoptotic cell death within 14 d (Berkelaar et al., 1994). After injury, activated glia, both in the retina and in the optic nerve, produce pro-regenerative neurotrophic factors (Hauk et al., 2008), indicating that the glial population also has some capacity for supporting axon regeneration in the adult mammalian CNS. However, reactive astrocyte and other glial cell-mediated remodeling of the ECM (Fisher et al., 2005; Lewis et al., 2010; Luna et al., 2010; Kayama et al., 2011) ultimately produces a glial scar containing proteoglycans (Silver and Miller, 2004), including neurocan, brevican, phosphacan, and versican (Jones et al., 2003; Butt et al., 2004), and myelin-derived molecules that inhibit axon growth in CNS tissues (Trimmer and Wunderlich, 1990; Schwab, 2010). Thus, in developing new regenerative therapies for CNS injuries, a more comprehensive “tissue-level” approach is required that includes modulating glial cell activation and ECM remodeling to suppress scarring.

Combinatorial approaches, targeting one or more axon growth-inhibiting factors by molecular and/or genetic manipulations, can improve RGC survival and increase axon regeneration in the visual system (Kurimoto et al., 2010). In optic nerve crush studies, full-length axon regeneration was reported in mice treated with combinatorial therapies that increase intrinsic axon regeneration potential, indicating that injured RGC axons can regenerate through the “host” optic nerve to reinnervate the brain. Though the percentage of neurons that regenerated sufficiently to reinnervate the brain was low, encouragingly, some functional recovery was reported (de Lima et al., 2012). Additionally, studies using peripheral nerve grafts and bioengineered neural bridges have shown that transected RGC axons can also regenerate over long distances through nonhost tissues (Berry et al., 2008) and biomaterials (Wittmer et al., 2011). In some cases, transected RGC axons regenerated sufficiently to reinnervate the brain. However, these studies also reported that a low percentage of axons regenerated successfully (Bray et al., 1987). Instead of trying to target numerous growth-inhibiting factors, which may lead to a never-ending series of complications due to off-target effects, or relying on foreign tissue or bioengineered neural bridges, which may not be compatible in the long term and are difficult to envision routing properly to the numerous visual centers in the brain, a more logical approach may be to treat CNS injury at the tissue level by altering the default healing response to injury.

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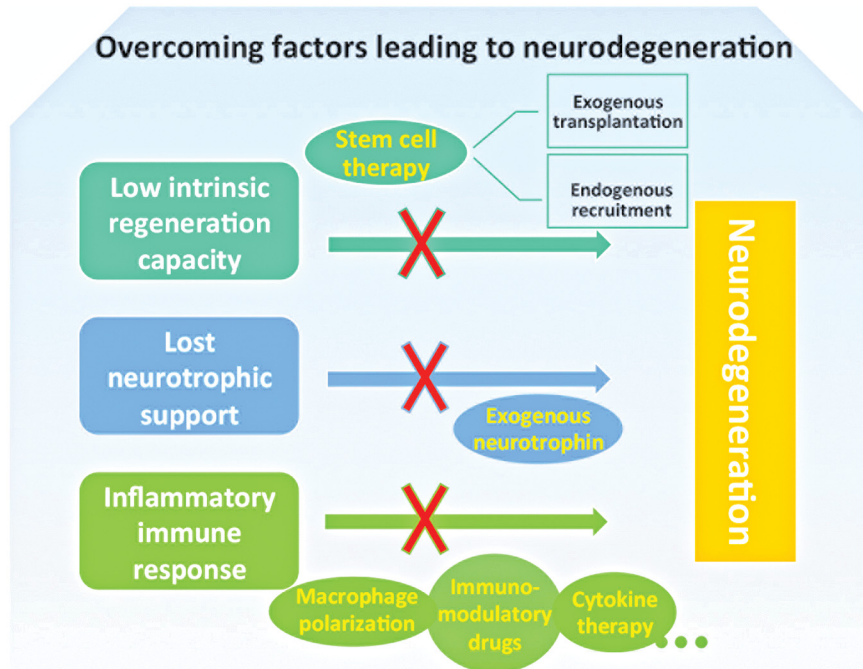


Figure 1. Numerous barriers must be overcome to prevent neural degeneration. The low intrinsic regeneration capacity of RGCs, lost neurotrophic support, and an inflammatory immune response are three major factors leading to neurodegeneration. Therapies overcoming these barriers are showing promise in preclinical and clinical models, including stem cell delivery from both exogenous and endogenous sources; neurotrophin delivery; and immunomodulatory therapies using macrophage polarization, immunomodulatory drugs, and cytokines.

The innate immune response and CNS regeneration

The default healing response in the CNS is closely linked to the timing and nature of the innate immune response to injury (Koh and DiPietro, 2011; Godwin et al., 2013). After CNS trauma, damaged and dying cells release chemokines and other molecules (Popovich and Longbrake, 2008; Brinkmann and Zychlinsky, 2012) that modulate multiple innate immune system cells, including neutrophils (Schnell et al., 1999; Donnelly and Popovich, 2008), macrophages (Soares et al., 1995), and microglia (Hains and Waxman, 2006), among others. Studies from animals that can regenerate CNS tissues, including retina, optic nerve, and brain, indicate that the temporal and spatial organization of macrophage phenotypes is a critical determinant in the overall healing response (Shechter et al., 2009, 2013). For example, in vertebrates like the axolotl, the temporal and spatial patterning of macrophage phenotypes appears to determine whether injured tissues are repaired functionally or whether scar tissue is formed (Godwin and Rosenthal, 2014).

Macrophages and microglia, the resident macrophage-like cells in the CNS, alter their phenotypes along a spectrum, ranging from the classically activated proinflammatory, M1-like phenotype to the alternatively activated anti-inflammatory, M2-like phenotype (Martinez and Gordon, 2014). This spectrum of phenotypes and their spatial and temporal ratios to one another play distinct roles in the healing process in most vertebrates, determining whether a tissue forms scar tissue or whether a

tissue remodels to preserve or restore function. M1-like and M2-like macrophages can generally be distinguished by assessing cytokine, receptor, and enzyme expression levels. Classic M1-like markers include, but are not limited to, proinflammatory markers like interleukin (IL)-1 β , IL-6, IL-12, tumor necrosis factor- α , inducible nitric oxide synthase, and C-X-C motif chemokine 10, while alternatively activated M2-like markers include transforming growth factor- β (TGF- β), IL-10, IL-1 receptor antagonist, arginase-1, CD206, and CD163 (Kawamura et al., 2009; Hao et al., 2012; Martinez and Gordon, 2014). Of note, M1- and M2-like markers can vary significantly depending on species, tissue, or organ, and the nature of the injury or pathogen. Thus, careful consideration of multiple markers must be used to identify macrophage phenotypes. Generally, proinflammatory M1-like cells produce high levels of oxidative metabolites (e.g., nitric oxide and superoxide) and proinflammatory cytokines, which are essential for host defense and tumor cell killing (Sica and Mantovani, 2012). However, these factors also cause secondary tissue damage, expand the injury area, and contribute to increased scarring (Mantovani et al., 2004). In contrast, M2-like macrophages are generally thought to be anti-inflammatory and promote functional tissue remodeling (Kigerl et al., 2009). Immunodepleting M1-like macrophages in adult mammals can reduce scarring and improve tissue preservation and functional outcomes. Conversely, cytokines like IL-4 or IL-13 promote an M2-like phenotype (Mokarram et al., 2012), which is thought to reduce tissue-destructive inflammation and to increase

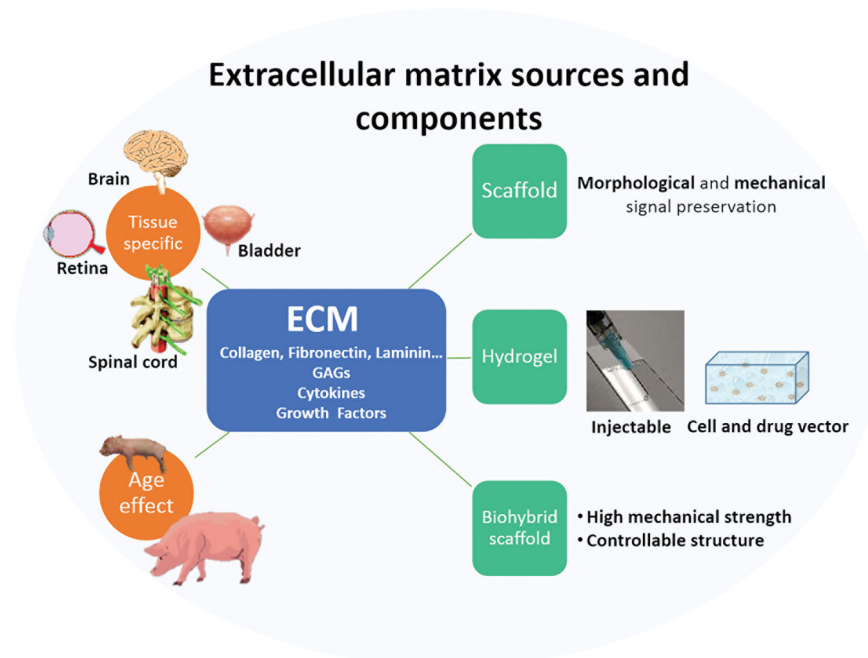


Figure 2. ECMs can be derived from different animal tissues or organs, each with a unique complement of proteins, carbohydrates, extracellular matrix molecules, growth factors, and cytokines. The ECM is a flexible platform that can be used as a natural material bioscaffold, an injectable hydrogel, or combined with polymeric materials to form biohybrid devices with controllable mechanical and biochemical properties. Each form can be augmented with cells or other bioactive molecules to improve the healing response.

functional tissue remodeling (Sica et al., 2006). In CNS tissues, M1 macrophages are neurotoxic and possess only moderate axon growth-promoting effects (Kigerl et al., 2009), whereas M2 macrophages are non-neurotoxic and can promote long-distance axon growth, even in the presence of growth-inhibitory molecules like chondroitin sulfate proteoglycans (CSPG) or myelin (Kigerl et al., 2009). The M2 phenotype has been implicated in tissue repair through cytokine secretion that in turn supports new ECM deposition and tissue remodeling during the wound-remodeling stage (Mosser and Edwards, 2008). Thus, after CNS injury, immunomodulatory therapeutics designed to positively modulate the ratio between M1-like and M2-like macrophages are a logical approach to improving CNS repair.

Extracellular matrix technology

How can we modulate microglia and macrophage phenotypes to promote a more favorable healing outcome in CNS tissues? Extracellular matrix technology is an attractive candidate. Naturally derived ECM bioscaffolds have been shown to modulate the innate immune response in wide-ranging applications throughout the body. ECM technology uses ECM bioscaffolds derived by decellularizing various healthy mammalian tissues or organs primarily from porcine or equine sources (Gilbert et al., 2006; Badylak, 2007; Fig. 2). ECM bioscaffolds maintain many of the bioactive molecules specific to the native tissue, including collagens, glycosaminoglycans, laminins, and growth factors (Crapo et al., 2012; Turner and Badylak, 2013), that are unavailable in synthetic materials. When properly prepared, ECM does not produce an adverse

immune response (Keane et al., 2012) and is highly translatable clinically. Over 4 million patients have been treated with >60 Food and Drug Administration-approved, ECM-based products used to treat injuries in varied tissues, including skin, heart, esophagus, bladder, muscle, bone, and peripheral nerves, among others (Badylak et al., 2005; Badylak, 2007; Valentin et al., 2010; Wolf et al., 2012). However, few initial studies have analyzed whether ECM technology can modulate the default healing response in the brain or spinal cord (Liu et al., 2011; Bible et al., 2012; Zhang et al., 2013), and applying ECM technology to retinal or to optic nerve injuries has been virtually unexplored.

ECM bioscaffolds can modulate several components of the default healing response relevant to constructive CNS tissue remodeling. For example, porcine small intestine ECM (SIS-ECM) bioscaffolds induce angiogenesis in an esophageal resection and repair model in dog (Badylak et al., 2000), and both angiogenesis and neurogenesis in a murine model of volumetric skeletal muscle loss (Sicari et al., 2012b). SIS-ECM bioscaffolds can also promote innervation in a rodent abdominal wall reconstruction model (Agrawal et al., 2009). ECM degradation products derived from digesting urinary bladder ECM can direct both progenitor cells and resident tissue-derived cells to repopulate the injury site in a mouse model of digit amputation (Agrawal et al., 2011, 2012). Decellularized peripheral nerve ECM can stimulate axon regeneration in the rodent sciatic nerve (Vasudevan et al., 2014) as well as in human clinical studies in part by stimulating Schwann cell migration and myelination (Karabekmez et al., 2009; Cho

et al., 2012). ECM-based hydrogels can reduce glial activation in rodent CNS trauma models (Lin et al., 2009; Khaing et al., 2011) with reports of improved neurologic function (Huang et al., 2012). Finally, ECM bioscaffolds also possess antimicrobial activities (Brennan et al., 2006; Medberry et al., 2012), which are critical in any successful wound-healing scenario.

However, modulating the innate response of the immune system to injury is increasingly recognized as the critical factor necessary to bias the default healing response toward site-appropriate, functional tissue remodeling. ECM-derived factors are hypothesized to modulate the innate immune response by regulating the spatial and temporal ratios between M1-like and M2-like macrophage and microglia phenotypes. Naturally derived ECM bioscaffolds have been shown to increase M2-like, pro-regenerative macrophages at the ECM implantation site in muscle defect models based on both immunohistochemical and quantitative PCR for M1-like and M2-like macrophage markers (Badylak et al., 2008; Brown et al., 2009, 2012). However, whether ECM can similarly regulate M1/M2 phenotypes in infiltrating macrophage and resident microglia, which differ considerably from monocyte-derived macrophages (Cherry et al., 2014), in the CNS is largely unknown. Depleting or inhibiting M1 macrophage activity has long been recognized as being neuroprotective in the CNS (Giulian and Robertson, 1990; Gris et al., 2004). In a rodent traumatic brain injury (TBI) model, urinary bladder matrix (UBM) injected into the brain did not detectably activate microglia or astrocytes, while decreasing the lesion volume and increasing functional recovery (Zhang et al., 2013). In a rodent spinal cord injury model, M2-like macrophage-derived factors were shown to reduce astrocyte activation, which in turn reduced M1-like macrophage infiltration via a putative feedback mechanism (Haan et al., 2015). Since the glial scar contains inhibitory molecules like CSPGs, which not only prohibit axon regeneration (McKeon et al., 1999) but also appear to polarize macrophages toward an M1 phenotype (Bartus et al., 2014; Didangelos et al., 2014), the glial scar appears to promote proinflammatory signaling indefinitely at the lesion site (Kigerl et al., 2009). Thus, the antigliotic and immunomodulatory properties of naturally derived ECM bioscaffolds should be further explored in the CNS as a preemptive strategy for reducing scarring.

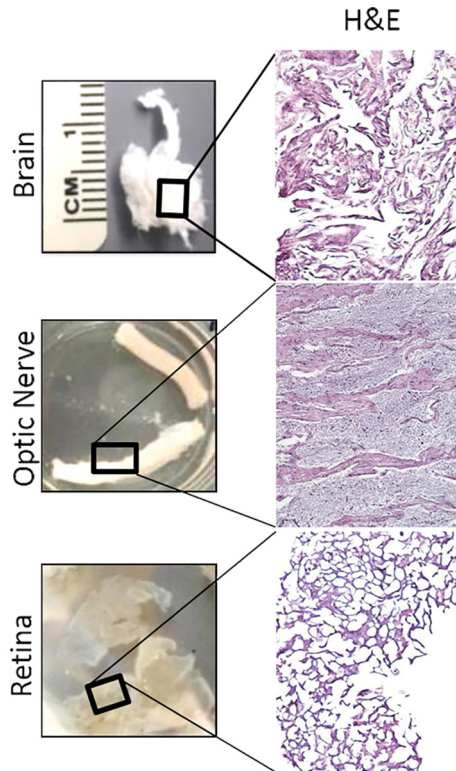
How do ECM bioscaffolds modulate the default healing response at a signaling level? ECM-induced changes in the default healing response are attributed to both chemotactic and chemotrophic factors, released during ECM degradation (Reing et al., 2009), that direct site-appropriate cellular migration and differentiation. In turn, appropriately differentiated cells feedback positively by exerting site-appropriate changes in the extracellular matrix in a process termed “dynamic reciprocity” (Bissell and Barcellos-Hoff, 1987; Berthiaume et al., 1996; Nelson and Bissell, 2006). After ECM is applied *in vivo*, ECM bioscaffolds are rapidly invaded and degraded by macrophages and other immune cells. During degradation, factors are released, including growth factors that contribute to healing, like VEGF (Sage, 1997; Hodde et al., 2001), TGF- β ,

PDGF, BMP4, and bFGF (McDevitt et al., 2003; Badylak, 2004). Recent studies have demonstrated that biologically active ECM degradation products are released from collagen, laminin, and fibronectin molecules (Davis et al., 2000; Adair-Kirk and Senior, 2008; Brennan et al., 2008; Crisan et al., 2008; Reing et al., 2009) and from angiogenic proteins (Sage, 1997; Hodde et al., 2001). These so-called “matricryptic peptides” can recruit endogenous stem cells and direct their migration (Agrawal et al., 2011), proliferation (Reing et al., 2009), and differentiation into site-appropriate cell types. For example, peptides generated by enzymatic degradation of urinary bladder ECM identified a C-terminal telopeptide of collagen III α that can direct stem cell chemotaxis *in vitro* and attract Sox2⁺/Sca1⁺/Lin⁻ progenitor cells *in vivo* in a mouse digit amputation model (Agrawal et al., 2011). Moreover, in a dog musculoskeletal model, ECM-derived scaffolds have been shown to recruit CD133⁺ myogenic progenitor cells (Turner et al., 2012) as well as Sca1⁺/PW1⁺ interstitial muscle stem cells (Perniconi et al., 2011). Whether similar mechanisms can direct CNS stem cell populations remains to be determined but is an ongoing area of investigation.

Both age and tissue type are important factors with ECM-derived from younger, tissue-matched sources, often achieving increased progenitor cell recruitment and enhanced ECM-mediated alteration of the default immune response toward a pro-repair M2 macrophage phenotype (Brennan et al., 2008; Sicari et al., 2012a). Thus, tissue-matched ECM bioscaffolds, like porcine brain, optic nerve, or retinal ECMs, may be good candidates for modulating the innate immune response in CNS-specific, brain, spinal cord, retinal, or optic nerve injuries. Furthermore, recent advances in decellularization techniques have permitted ECM to be derived from delicate tissues previously not decellularizable using established protocols, including fetal brain, optic nerve, and retina (Fig. 3). The ability to use hydrogels derived from fetal, tissue-matched sources, particularly in delicate CNS tissues like the retina, is an exciting and open area for investigation. Preliminary *in vitro* studies indicate fetal, tissue-specific ECMs can increase retinal ganglion cell survival and axon regeneration significantly over adult tissue-derived versions (Fig. 4).

Modulating inflammation is critical to a positive outcome in CNS tissues like retina and optic nerve

Inflammation plays a central role in the healing outcome and in the rate of disease progression. Inflammation exerts both positive and negative effects on RGC regeneration. After incisional or penetrating trauma to the retina, cells die in all retinal layers adjacent to the wound (Sipperley et al., 1978; Turner et al., 1986). Similarly, all photoreceptors adjacent to the wound die by apoptosis or programmed necrosis. Retinal cell death is accompanied by the rapid onset of glial activation (Yoshida et al., 1995; Fisher et al., 2005). Within 30 min of injury, cell cycle-related transcription factors that regulate astrocyte proliferation, like c-Fos and Jun-B, are upregulated (Fisher



Fetal ECM can be derived from porcine sources.

Figure 3. Images and H&E staining of different decellularized CNS ECMs derived from fetal porcine tissues.

et al., 2005). Within 3 d, glial fibrillary acidic protein and proliferating cell nuclear antigen, markers of activated and proliferating glial cells, are upregulated (Yoshida et al., 1995; Vazquez-Chona et al., 2004). Activated glia in the retina and in the optic nerve produce multiple pro-

regenerative neurotrophic factors (Hauk et al., 2008; Ahmed et al., 2010). However, reactive astrocytes also contribute to injury-induced ECM remodeling; altered synaptic connectivity in the retinal layers; and altered photoreceptor cellular organization, function, and synaptic connectivity (Fisher et al., 2005; Lewis et al., 2010; Luna et al., 2010; Kayama et al., 2011). Various proinflammatory stimuli, including lens injury, intravitreal injection of zymosan (macrophage activator) or stem cells, and intravitreal peripheral nerve grafting, can all reduce injury-induced RGC death (Berry et al., 1996; Leon et al., 2000; Yin et al., 2003; Mead et al., 2013), presumably by modulating the nature of the inflammatory response. For example, after lens injury and intravitreal zymosan injection, retinal astrocytes and Müller cells release CNTF, which supports axon regeneration (Muller et al., 2007). Immunomodulation combined with endogenous or exogenous growth factors can enhance RGC regeneration synergistically (Kerschensteiner et al., 2003; Lorber et al., 2008). After incision-induced trauma to the retina, some types of inflammation appear to reduce retinal cell death and increase RGC axon regeneration (Berry et al., 2008). Immunosuppressive agents like corticosteroids have also been used to treat intermediate and posterior segment uveitis with some success. Corticosteroids can reduce inflammation and alleviate other structural complications. However, complete suppression of the immune system leads to secondary complications (Thiyagarajan et al., 2013) that may actually prohibit long-term recovery in the CNS. In general, inflammation due to retinal trauma promotes an M1-like phenotype, which decreases RGC survival and increases scarring (Cruz-Guilloty et al., 2013; He and Marneros, 2013), emphasizing the importance of identifying the type and the scope of the inflammatory response, as well as highlighting the need for readily available and versatile immunomodulatory therapeutic platforms.

Can ECM-based immunomodulatory devices fill this need in the CNS, for example by slowing or halting retinal disease progression? Increasingly, microglial activation is implicated in retinal disease pathogenesis, including glaucoma (Yuan and Neufeld, 2001), diabetic retinopathy (Zeng et al., 2008), age-related macular degeneration (AMD; Gupta et al., 2003), and retinitis pigmentosa (RP; Gupta et al., 2003), among others. Transplantation of human induced pluripotent stem cell-derived retinal cell types has been proposed as a potential treatment for AMD and RP. However, this treatment strategy needs to be optimized with regard to characterizing and preparing donor cells (Ramsden et al., 2013). Current clinical immunotherapies can inhibit microglial activity (Yrjänheikki et al., 1998). However, such strategies can also disrupt microglial regulation of CNS homeostasis (Edan et al., 2013). Thus, a more biocompatible immunotherapy like ECM may be advantageous under certain conditions alone or as an adjunct therapy. For example, in AMD, macrophages penetrate the interphotoreceptor matrix and polarize toward an M1-like phenotype, which is recognized as a key factor in dry AMD pathogenesis (Cruz-Guilloty et al., 2013). In retinal diseases, can ECM hydrogels be used to modulate microglial activation di-

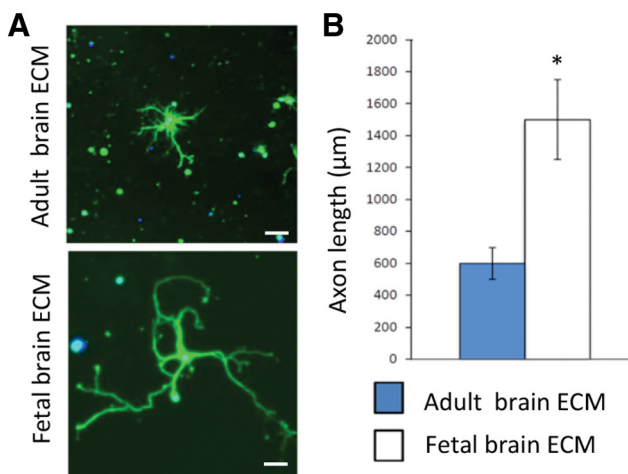


Figure 4. RGCs grow longer processes in ECM derived from younger, homologous tissue sources. **A**, Fluorescence images of purified primary rat RGCs cultured in adult or fetal brain ECMs. **B**, Quantification: $p < 0.001$, ANOVA.

rectly or in combination by delivering other therapeutics in a natural, injectable, biodegradable device? And, if so, can sustained modulation be achieved in various diseases?

ECM technology can mitigate secondary trauma

Primary mechanical injuries are often followed by additional tissue destruction and expanded scarring due to the inflammatory response. In the visual system, secondary ocular trauma in the retina or in the optic nerve can rapidly increase the injury area and lead to increased vision loss. One of the most detrimental injury conditions is during warfare, where significant delays in ocular treatment are common. In modern warfare, blast injuries are the most common wounded-in-action injuries accounting for ~60% of all injuries as of July 2009, with up to 40% of blast injuries expressing concomitant eye injuries. To treat ocular trauma due to penetrating injuries and intraocular foreign bodies, the current standard of care requires a vitreoretinal service and a microsurgical operating suite, which is generally not possible on the battlefield or in Level III combat support hospitals. In a combat support hospital, patients do receive an eye evaluation and primary surgical repair by an experienced ophthalmologist (Weichel and Colyer, 2008), often within hours of injury. However, in cases where a microsurgical suite and a vitreoretinal service are required, patients typically experience a minimum 72–96 h delay before evacuation. These delays are during a critical period in the healing process since secondary inflammatory trauma can increase progressively and is often more damaging than the primary trauma. For example, secondary inflammatory responses to penetrating injuries can contribute to endophthalmitis, which can lead to vision and even eye loss (Lemley and Han, 2007), or proliferative vitreoretinopathy (PVR), which can result in progressive retinal traction, tears, and retinal detachment (Charteris, 1995). The molecular cascades leading to PVR are detectable within hours in rodent models of PVR (Wen et al., 1995; Ozaki et al., 2000; Penn et al., 2006), again emphasizing the need for therapies to suppress secondary inflammation, like ECM-based devices, which are safe, derived from renewable resources, and relatively inexpensive, and can be tailored to the nature and scope of the injury.

ECM hydrogels are a highly tunable biologic platform

To address this problem, ECM hydrogel technology is being developed in two platforms designed to rapidly mitigate secondary trauma in varied injuries. The first platform is an injectable hydrogel (Medberry et al., 2013) that can be injected into or around the eye, whereas the second platform is a convenient ECM and polymer biohybrid sheet (Hong et al., 2011). These technologies are designed to stabilize retinal or optic nerve injury, reduce scarring, and increase the potential for vision preservation. Both platforms are tunable, both mechanically and biochemically, to deliver drugs, cells, or other therapeutics in a biocompatible, immunomodulatory platform.

ECM hydrogels

ECM-based hydrogel scaffolds are advantageous because of their potential for minimally invasive delivery. Applying ECM technology to injured neural tissues has been limited, as previously reviewed (Meng et al., 2014), particularly in the CNS where intrinsic regenerative ability is low (Goldberg et al., 2002). However, a number of studies in spinal cord have shown that acellular ECM matrices can integrate within host spinal cords after traumatic brain injury and even improve motor function (Li et al., 2012; Zhang et al., 2012; Liu et al., 2013). UBM has been shown to promote constructive cellular responses to and neuroprotection of injured brain tissues (Zhang et al., 2013). After injection into healthy brains, UBM did not increase microglia accumulation or astrocyte activation, or promote neuronal degeneration. After TBI in rats, UBM treatment reduced lesion volume and myelin disruption, and improved vestibulomotor function. However, cognitive recovery was undetected over the time points analyzed. Recently, Wang et al. (2013) reported similar results following TBI, showing that UBM decreased sensorimotor skill loss, indicating that ECM hydrogels may be a viable treatment option in CNS tissues. In cases of penetrating ocular trauma, local ECM hydrogel injection provides a biocompatible, tunable gel for filling acellular injury-induced defects, recruiting and directing endogenous stem cell localization and differentiation, and modulating the immune system to promote positive tissue remodeling.

ECM biohybrid bioscaffolds

Biohybrid scaffolds confer several advantages to ECM hydrogels by combining the mechanical and biochemical tunability of an electrospun polymer sheet with the biologic properties of ECM (Hong et al., 2011; Fig. 5). In regenerative medicine approaches that require bioscaffolds with a higher tensile strength, hydrogels are disadvantageous due to rapid degradation rates and poor mechanical properties. The mechanical strength of ECM hydrogels can be improved by chemical crosslinking (Baiguera et al., 2014). However, crosslinking changes ECM composition, leading to increased risk of inflammation (Valentin et al., 2009), altered degradation rates and products, and altered biological efficacy and effects. Combining ECM hydrogels with a synthetic biocompatible and biodegradable polymer has been shown to be a viable option (Stankus et al., 2008; Yoon and Kim, 2010). Hong et al. (2011) used dual-stream electrospinning to blend synthetic poly(etherurethane urea) (PEUU) polymer and urinary bladder matrix. The resulting ECM biohybrid scaffolds have tunable degradation properties and tunable mechanical properties. For example, electrospun fibers can be aligned to direct axon growth. Kador et al. (2014) created a scaffold with aligned fibers by electrospinning, which guided retinal ganglion axon growth directionally. By integrating ECM hydrogels, biohybrid scaffolds possess higher biocompatibility compared with purely synthetic scaffold materials. In non-CNS models, biohybrid scaffolds have been shown to promote constructive tissue remodeling in ligament, heart, and body

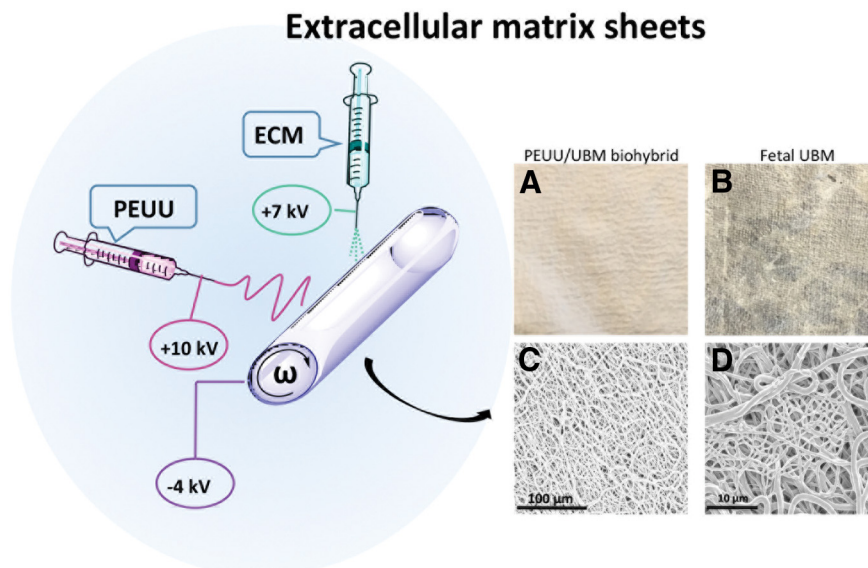


Figure 5. Extracellular matrix in two sheet forms. ECM biohybrid sheets can be made by dual-stream electrospinning. PEUU and ECM electrospinning setup consists of two syringe pumps and two high-voltage power supply units (not shown). A high positive voltage (+7–10 kV) is used to charge the steel capillary containing the polymer or ECM solution, and a high negative voltage (−4 kV) is used to charge the stainless steel mandrel (ω). The mandrel is rotated at 200 rpm with a slow lateral translation over a distance of 15 cm. **A**, Yielding a tubular, uniform PEUU/ECM sheet. **B**, Fetal urinary bladder sheet after vacuum pressing. **C**, **D**, Scanning electron microscopy showing the random fibers in the PEUU/ECM biohybrid wrap.

wall (Hong et al., 2011; Thayer et al., 2014; Jahnavi et al., 2015). In nervous system tissue repair scenarios, the ability to readily form biohybrid scaffolds into a suturable nerve wrap or a patch to protect exposed tissues makes ECM biohybrid scaffolds attractive devices for both mechanical and biochemical support.

ECM hydrogels for cellular transplantation

ECM hydrogels provide a tissue-specific, naturally derived platform for delivering transplanted cells in an injectable platform. Transplanting purified retinal progenitor cells or neural stem cells (NSCs) has emerged as a promising therapy for preserving visual function. Intravitreal or optic nerve injections of RGCs, RGC progenitors, or stem cells are minimally invasive and have been shown in some neurodegenerative disease models to be neuroprotective (Klassen et al., 2008; Eveleth, 2013). Transplanted RGCs or stem cells are hypothesized to slow retinal degeneration by modulating multiple prosurvival pathways simultaneously via locally secreted neurotrophic factors and/or via modulation of the intraocular microenvironment (Miyata et al., 2005; Hertz et al., 2014). And clinical trials have been performed to test these hypotheses (Ning et al., 2011). However, RGCs injected into the vitreous integrate randomly without the correct spatial and cellular organization. Moreover, although transplanted RGCs can extend long axons, their orientation is generally not directed toward the optic nerve head, a prerequisite for restoring RGC connectivity to the brain. Transplanting sheets of fetus-derived retinal progenitor cells subretinally can restore some vision in both animal models and humans. In Phase II clinical trials, visual acuity was improved in pa-

tients with retinitis pigmentosa or macular degeneration (Seiler and Aramant, 2012), demonstrating that cellular transplantation strategies can preserve retinal function in cases where the ganglion cell connectivity to the brain is intact. Moreover, retinal sheets hold promise for replacing RGCs with the proper cellular organization in the retina. However, similar to single-cell RGC transplantation, retinal sheets cannot replace lost RGCs with the correct retinal and optic nerve organization, and thus cannot currently restore vision loss due to lost RGCs. By using CNS tissue-specific ECM hydrogels, stem or progenitor cells can be encapsulated and then injected precisely within a biocompatible matrix, which may offer greater potential for integration and preservation of function.

In other cases, ECM biohybrid sheets may provide a better option for delivering cells. For example, mesenchymal stem cells (MSCs) can modulate immune and inflammatory effects after injury in both the CNS and peripheral nervous systems (PNS). MSC delivery into the injury site can provide anti-inflammatory, immunomodulatory, and neuroprotective benefits. For example, in dogs with acute spinal cord injury, the injection of MSCs significantly improved functional recovery (Penha et al., 2014). However, further studies have shown these injections cause additional injury due to needle penetration, spinal cord motion during injection, creation of intraparenchymal pressure gradients, and hydrodynamic dissection, instilling deformed cell masses and possible cord ischemia (Donnelly et al., 2012). Other studies, using scaffolds, have shown that fibrin sheets incorporating MSCs demonstrate longitudinal alignment of MSCs and infiltration of host neurites, which are correlated with further improvement of functional outcomes (Hyatt et al., 2014). Several mechanisms

Using ECM to promote positive tissue remodeling

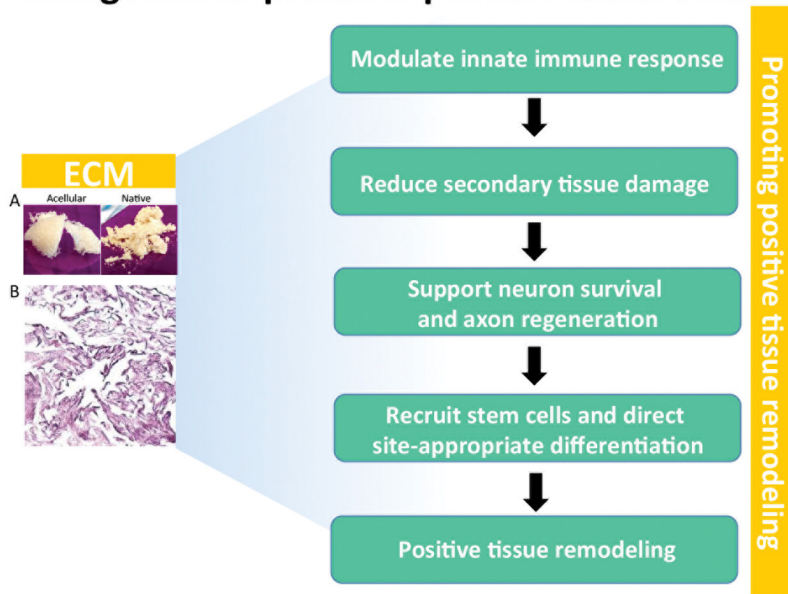


Figure 6. ECM technology can promote positive tissue remodeling by modulating several factors that contribute to the default healing response in the CNS.

have been proposed for the improved axonal regeneration seen after injecting MSCs, including paracrine effects due to the release of trophic factors, including BDNF, NGF, VEGF, FGF-2, TGF- β , and interferon gamma-1 (Mead et al., 2014). By incorporating mesenchymal stem cells into ECM biohybrid sheets, the neuroprotective potential of stem cells is combined with the immunomodulatory benefits of ECM. Moreover, incorporating stem cells into a polymer with tunable biodegradability provides more control over restricting the cells to a defined location, like subretinal implantation or as an optic nerve wrap, while permitting the soluble factors released from both stem cell and macrophage degradation of the ECM components, which have been shown in some systems to have positive synergistic effects with regard to tissue repair (Liu et al., 2011)

Can tissue-specific ECMs provide a more efficacious platform for stem or progenitor cell differentiation and integration? Numerous studies have shown that ECM bioscaffolds can direct site-appropriate cellular differentiation from both endogenous (Horne et al., 2010) and mesenchymal stem cell (Wang et al., 2010) sources. There are numerous examples of tissue-specific ECMs directing tissue-specific cellular phenotype differentiation and function. For example, cartilage-derived ECM can promote chondrogenic differentiation of adipose-derived stromal stem cells (Wang et al., 2014), while cardiac ECM can direct cardiac myocyte differentiation (Xu et al., 2014). Acellular spinal cord scaffolds can promote positive tissue remodeling in spinal cord injury models, similar to MSCs (Liu et al., 2013; Xue et al., 2013). Methods for decellularizing spinal cord (Guo et al., 2010), brain (DeQuach et al., 2011), and optic nerve (Crapo et al., 2012) have been developed from adult and even from fetal sources (Fig. 3).

Acellular CNS ECM scaffolds retain neurosupportive proteins, growth factors in a three-dimensional scaffold. Compared with UBM, CNS-derived ECMs induced PC12 cell migration, while UBM inhibited migration (Crapo et al., 2012). Brain-derived ECM improved neurite growth of neural stem cells (Medberry et al., 2013) and neuronal differentiation of induced pluripotent stem cells. (Crapo et al., 2012). These studies suggest that ECM may provide tissue-specific advantages in CNS regenerative medicine applications and that ECM scaffolds in general may aid functional recovery after retinal disease.

Non-tissue-specific ECMs can also promote positive CNS repair

Acellular muscle ECM has been used to treat spinal cord hemi-sections in rats. With the help of its parallel tubule structure, sprouting axons grew the full length of the scaffold in a strikingly parallel and linear fashion (Zhang et al., 2012). Loaded with amniotic epithelial cells, acellular muscle scaffolds further promoted nerve fiber sprouting and remyelination, resulting in more functional recovery (Xue et al., 2013). Acellular vessels are also a candidate for nerve regeneration scaffolds. Acellular vessels were used as nerve conduits connecting the two stumps of injured peripheral nerves (Sun et al., 2011). Acellular PNS scaffolds may also be a good choice for optic nerve repair. Sciatic nerves were decellularized with different methods (Gao et al., 2014), and their function in nerve regeneration was evaluated (Li et al., 2012; Gao et al., 2014). Schwann cells can support RGC axon growth, possibly due to neurotrophic factor secretion and/or the neurotrophic cell receptors on the membrane of Schwann cells (Baehr and Bunge, 1989; Hu et al.,

2005). Treating ECM with neurotrophic factors can also improve their ability in CNS regeneration (Li et al., 2012).

Cellular viability and retinal integration are improved by using various hydrogel materials, including hyaluronic acid (Gao et al., 2012, Carey et al., 2014), Matrigel (Sharp and Archer, 2015), alginate (Yang et al., 2015), collagen, (Cruz-Guilloty et al., 2013), and fibrin (Meng et al., 2014). Brain ECM hydrogels promoted neuronal differentiation of induced pluripotent stem cells better than Matrigel-coated surfaces (DeQuach et al., 2011). DeQuach et al. (2011) produced brain ECMs and found that neurons derived from human induced pluripotent stem cells plated on the brain matrix express neuronal markers and assume neuronal morphology. Crapo et al. (2014) showed that CNS-derived ECMs show higher ability in inducing neuron differentiation of neuronal stem cells compared with UBM. Liu et al. (2013) developed MSCs that loaded acellular spinal cord ECM, which promoted long-distance axon regeneration *in vivo*. Moreover, UBM hydrogel has been used as a carrier for NSCs and injected into rodent brain. The transplants reduced neuron/tissue loss and white matter injury, and also significantly ameliorated motor, memory, and cognitive impairments (Wang et al., 2013). Stem cells delivered by UBM hydrogels in a murine stroke model distributed uniformly throughout the lesion cavity instead of integrating into the host parenchyma. Better distribution was associated with better primitive tissue formation (Bible et al., 2012).

Conclusion

ECM-derived hydrogels are natural, biocompatible devices that can modulate inflammation, attract and direct stem cell proliferation and differentiation, and serve as a tunable platform for delivering an almost unlimited combination of genetic, molecular, and cellular therapeutic factors. ECM bioscaffolds have been shown to reduce inflammation and scarring while improving positive tissue reconstruction in tissues throughout the body. We hypothesize that tissue-specific hydrogels can do the same in CNS tissues generally as well as specifically within the retina and the optic nerve (Fig. 6). Ultimately, a highly defined and targeted approach is desired to treat CNS injuries with specificity. Thus, future studies should include further characterization of the bioactive components in the ECM, how various ECM components impact the innate immune response, and, in turn, how soluble and nonsoluble factors act on other cells within the CNS, including the glia, primary neurons, and progenitor and stem cells.

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