CHAPTER

Neural ECM mimetics 16

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Abstract

The consequence of numerous neurological disorders is the significant loss of neural cells, which further results in multilevel dysfunction or severe functional deficits. The extracellular matrix (ECM) is of tremendous importance for neural regeneration mediating ambivalent functions: ECM serves as a growth-promoting substrate for neurons but, on the other hand, is a major constituent of the inhibitory scar, which results from traumatic injuries of the central nervous system. Therefore, cell and tissue replacement strategies on the basis of ECM mimetics are very promising therapeutic interventions. Numerous synthetic and natural materials have proven effective both *in vitro* and *in vivo*. The closer a material's physicochemical and molecular properties are to the original extracellular matrix, the more promising its effectiveness may be. Relevant factors that need to be taken into account when designing such materials for neural repair relate to receptor-mediated cell–matrix interactions, which are dependent on chemical and mechanical sensing. This chapter outlines important characteristics of natural and synthetic ECM materials (scaffolds) and provides an overview of recent advances in design and application of ECM materials for neural regeneration, both in therapeutic applications and in basic biological research.

Keywords

Extracellular matrix, Neurodegeneration, Neuronal regeneration, ECM mimetic, Scaffold

1 ECM MIMETICS ON THE RISE

The loss of nerve cells is a general and major symptom of many disorders of the nervous system. After a traumatic injury, the differences in the regenerative response of affected neurons of the peripheral nervous system (PNS) versus the central nervous

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system (CNS) become obvious: While neurons of the PNS reveal a significant degree of regeneration after injury, such regenerative neuronal growth generally fails after an injury of the adult CNS although central neurons have some, but very limited, inherent regenerative capacity (David and Aguayo, 1981). The regenerative failure after CNS trauma and neurodegeneration, therefore, cannot be attributed to just one single cause, and the reasons for the difference in the regenerative responses of PNS and CNS are manifold (Ferguson and Son, 2011) including (i) the presence of myelin-associated inhibitors, (ii) a slower rate of degeneration of the distal segment of the injured fiber tract, (iii) a generally slower axonal growth rate, and (iv) the inhibitory influences of the glial and the extracellular environment. Neurodegeneration does, however, not only occur after CNS trauma. Neurodegenerative disorders like Alzheimer's disease, Parkinson's disease, stroke, multiple sclerosis, and amyotrophic lateral sclerosis result in the shared symptom of neuroinflammation, which eventually leads to the dysfunction or even the loss of neurons (Benowitz and Popovich, 2011; Cartier et al., 2005). Because of a general significant increase of the aging population and the consequence of an increased incidence of neurodegenerative diseases, the development of therapies, which may help to rescue and replace the affected cells and thereby preserve the associated functions, is of highest priority. The ECM is, therefore, an important target for the development of therapies for disorders of the CNS, which involve progressive neurodegeneration. Such interventions either directly target the inhibitory environment and its associated molecules, or they function on the basis of replacement strategies. For the latter, ECM mimetics (natural or synthetic matrix materials that mimic the characteristics of native ECMs) provide promising means for cell- or matrix-based therapeutic treatments.

The previous chapters have described the neural ECM and its role in a variety of healthy normal and injury-related processes in full detail. Chapters 16 and 17 discuss current therapies that directly target the neural ECM. However, if such respective therapies cannot be applied or are not efficient, e.g., in case of large tissue defects, or if *in vivo* differentiation is intended after cellular transplantation, it may be necessary to reconstruct the affected tissue. For example, via the application of suitable matrix mimetics, a growth-promoting microenvironment can be created. This chapter focuses on scaffold materials that are currently used as neural ECM mimetics in basic research and experimental therapies (Figs. 1–3).

2 THE EXTRACELLULAR MATRIX

A brief outline of the natural ECM will illustrate its complexity and suggest the difficulty and possible obstacles for the design of ECM mimetics: The ECM is composed of a three-dimensional meshwork of fibrillar proteins and glycosaminoglycans (GAGs) providing an interstitial matrix or basement membrane. The interstitial matrix consists of polysaccharide gels and fibrillar proteins, whereas sheetlike depositions of ECM are characteristic for basement membranes. The molecular ECM components are GAG-containing proteoglycans (chondroitin sulfate, heparan sulfate, and keratin sulfate), nonproteoglycans containing polysaccharide hyaluronic

Application of neural ECM mimetics	Cell-based	A-cellular
Biomedical research	Cell/tissue replacement, cell implantation, bioengineering	
Biomedical research	Bridging of tissue defects	Bridging of tissue defects
Biomedical research	Oxidative stress	
Biomedical research	Growth factor delivery	Growth factor delivery
Medical application	Minimization of secondary injury events	Minimization of secondary injury events
Medical application	PNS/CNS trauma	PNS/CNS trauma
Medical application	Neurodegenerative conditions	
Medical application	Neuroinflammation	Neuroinflammation

FIGURE 1

Possible applications of neural ECM mimetics. Cell-based and a-cellular scaffolds are suitable matrices for numerous strategies in both basic biomedical research and medical therapy.

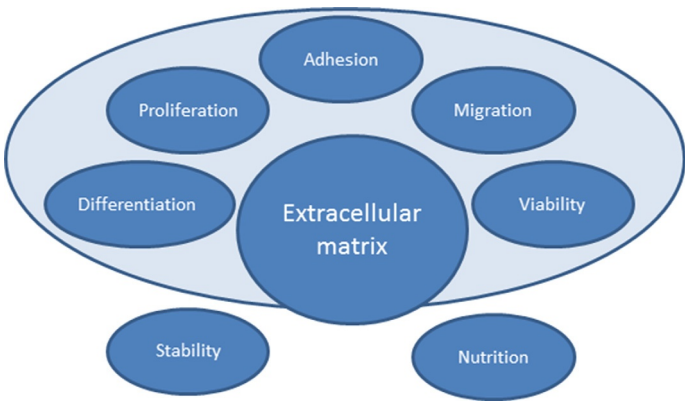

FIGURE 2

Basic functions of the natural extracellular matrix. One important general feature, which is exerted by the ECM, is the provision of a physically stable scaffold. The maintenance of ion homeostasis and the exchange of nutrients are vital for the correct function of cellular networks and tissues. ECM characteristics, which are especially important for cell–cell and cell–matrix interactions, respectively, and which are mediated by a delicate ECM meshwork, are highlighted by the light blue ellipse. These functions comprise the cellular processes of differentiation, migration, adhesion, proliferation, and viability.

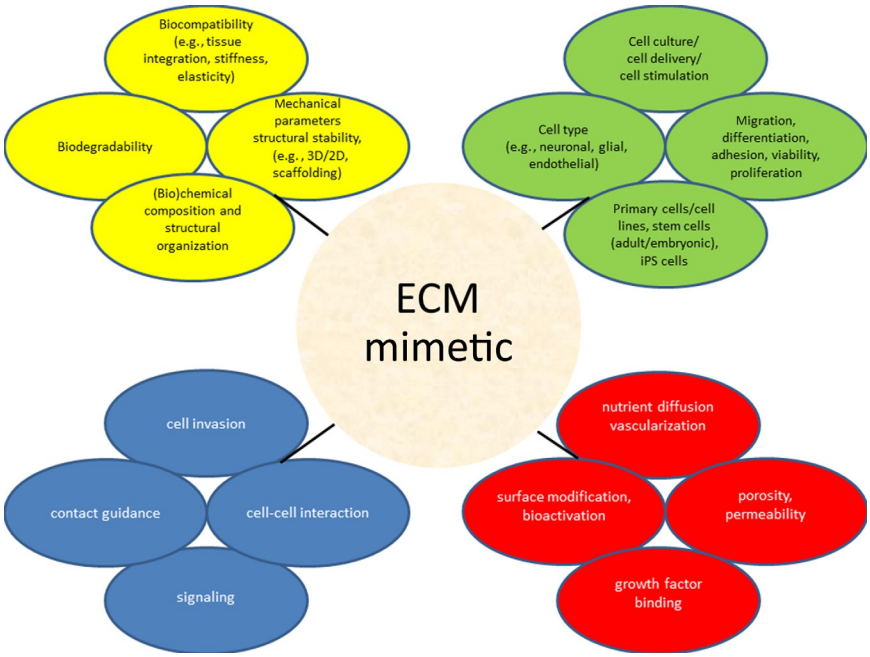

FIGURE 3

Requirements and considerations for the design of ideal neural extracellular matrix mimetics. The more closely an ECM mimetic resembles the natural ECM, the better it can exert the necessary functions. In addition to mechanical parameters, which strongly influence the cellular behavior and tissue stability, general features (yellow (light gray in print version)) of an ECM mimetic include its biocompatibility, biodegradability, and its general composition. Many characteristics, which are cell-specific (green (gray in print version)), would be considered for neural ECM design in cell replacement and general cell implantation strategies, for example, the cell type, whereas other basic functional requirements also apply for the general cellular behavior in a matrix (e.g., migration and differentiation). The different requirements certainly intermingle in an ideal neural ECM mimetic and, therefore, should not be considered independently of each other. For instance, cell–cell interactions are very important cell-related functions, but they are also vital for the structural organization, biocompatibility, etc. and for complex functions (blue (dark gray in print version)) such as signaling or contact guidance. Finally, synthetic and natural ECM mimetics can be designed and "tuned" to allow very precise functions (red (black in print version)). This can be achieved by the addition of bioactive groups to a scaffold material (surface modification) or by incorporating different degrees of porosity. Thereby, vascularization and subsequently nutrient exchange of an extracellular matrix can be influenced.

acid (HA), fibers of collagen or elastin, and connecting proteins such as fibronectin or laminin. Due to their water-attracting features, proteoglycans keep the ECM well hydrated. Furthermore, they aid in the entrapment and the storage of growth factors. Collagens, the most abundant proteins of the ECM, are mostly fibrillary proteins that provide structural and tensile support for cellular bonds. Exceptions are collagens

of the nonfibrillary type including collagen IV, which forms the sheetlike meshed, nonfibrillar network of the basement membrane. The important functions that are common to ECMs in general are cell adhesion, cell communication, and cell differentiation. ECMs provide structural stability to tissues. The formation of ECM is essential for several vital processes, such as wound healing, growth, and fibrosis. Cell–ECM interactions are highly dynamic bidirectional processes, which require a very complex temporal and spatial coordination of signaling, receptor-mediated transmission, and regulation of gene expression for their correct function. The three main effectors in a native ECM microenvironment are insoluble hydrated macromolecules, soluble macromolecules, and surface proteins of neighboring cells (Lutolf and Hubbell, 2005). The molecular interaction of a cell with these effectors will determine its ultimate fate, i.e., differentiation, proliferation, migration, apoptosis, or other specific functions.

CNS trauma research models (e.g., experimental spinal cord injury) are useful in order to explore the suitability of different materials as possible ECM mimetics *in vivo*: large tissue defects that result from primary and secondary injury events (e.g., inflammation, scarring, or cavity formation) and the pathological changes in the native ECM require the removal and/or the reconstruction of the affected tissue in order to restore tissue continuity. A variety of scaffold-based strategies exist in the field of experimental CNS trauma, which involve either cellular transplantation or the implantation of matrix materials as bridging substrates or a combination of both. *In vitro* cell culture experiments often focus on the survival, migration, and differentiation of the cells in a suitable matrix. *In vivo* cell transplantation approaches often target similar effects, but the complex and mostly inhibitory environment requires additional considerations.

3 INTERACTION OF ECM SCAFFOLD AND HOST ECM

The biocompatibility or the interaction of an artificial scaffold material with host tissue can be modulated via the surface characteristics of the material. Different conditions require different treatments and, therefore, different matrix characteristics. While for cell replacement strategies the supply with factors that mediate cellular differentiation, migration, and survival is of high priority, the degree of biodegradability is a very important issue in regenerative medicine because it can have a significant effect on the extent of tissue regeneration. However, biodegradation of implanted biomaterial can cause side effects on the surrounding host tissue (Sakiyama-Elbert et al., 2012). Toxic by-products might elicit immune responses or changes in the pH, which could weaken or even reverse any beneficial treatment effects. Therefore, the efficiency of biomaterials that come into consideration as ECM mimetics should ideally be investigated both *in vitro* and subsequently *in vivo*.

The most basic function of ECMs is the provision of a scaffold structure. For neural ECM mimetics, which are used as scaffold materials in regenerative medicine, it is further desirable that they promote angiogenesis and vascularization. Blood vessels are required for the provision of nutrients and removal of waste products,

processes that are vital for tissue regeneration (Owen and Shoichet, 2010). Angiogenesis and vascularization of the scaffold implant have been reported for numerous neural ECM biomaterials (Bakshi et al., 2004; Bramfeldt et al., 2010; Estrada et al., 2014; Plant et al., 1997; Woerly et al., 1999).

The presence and orientation of pores or channel structures in a matrix can guide the cellular growth (Sakiyama-Elbert et al., 2012). Highly complex conditions like spinal cord injuries require different molecular interventions or their combination to mediate neural cell adhesion and axon sprouting and extension. Multiple cell types and neuronal populations are affected by traumatic spinal cord injury, and therefore, possible therapeutic strategies are numerous.

Often, cells will adhere nonspecifically to unmodified ECMs. Bioengineering scaffold materials via incorporation of proteins or peptides on its surface can be used to design scaffolds with specific cell–scaffold interactions to influence the cellular behavior. Incorporation of specific peptide sequences, which promote cell adhesion, is a common way of designing functionalized ECM mimetics with the potential for enhanced cellular adhesion or better integration of the respective material (Choi et al., 2013; Dhoot et al., 2004; Plant et al., 1997).

The physical structure and rate of degradation of a neural ECM scaffold have a great impact on nervous tissue regeneration. If the material is degraded too fast, the damaged tissue may lack the necessary mechanical support that is required for regeneration. On the other hand, nondegradable materials provide a long-term structural stability but may be critical in regard to clinical applications since the implant will remain in the patient's body for an extended period of time or even permanently.

Optimal integration of ECM scaffold material will facilitate its effectiveness. It is, therefore, a high priority to minimize scarring or allergic reactions caused by the implanted material. Increased scarring responses can eventually result in an encapsulation of the foreign material from the adjacent host tissue (Sakiyama-Elbert et al., 2012; Stensaas and Stensaas, 1978). With respect to cell–matrix interaction, the hydrophobic surface of a potential neural ECM scaffold material can significantly decrease the attachment of cells (Sakiyama-Elbert et al., 2012). This effect may be desired for inflammatory cells or reactive astrocytes. But the overall prevention of cellular adhesion is an unwanted effect, which would negatively influence neural regeneration.

4 ECM MIMETICS

Recent advances in the design of materials, which imitate the microenvironment of the ECM, include nanofibrillar networks, artificial ECM networks, and synthetic polymers (Lutolf and Hubbell, 2005; Sadr et al., 2012; Tian et al., 2012; Tong and Yang, 2014). Depending on their designated purpose, these scaffold materials can be designed with different properties, regarding material type, ionic charge, general physicochemical properties, incorporated bioactivity (functionalization), addition of soluble factors, or biodegradability.

The guided cell growth that is provided by such materials is intended to facilitate the structural and the functional reorganization and regeneration of dysfunctional tissues. Therapeutic approaches, which make use of matrix- or scaffold-based strategies, can be divided into two general classes (Lutolf and Hubbell, 2005):

1. Cell-based therapies
2. Acellular therapies

Cell-based therapies imply the delivery of cells and may involve the use of bioengineered tissues (biohybrid scaffolds), whereas acellular therapies rather target cells of the healthy residual tissues *in situ*, which can be stimulated and directed by the matrix material in their growth or differentiation.

In the field of neurodegeneration, cell transplantation strategies are widely pursued in basic research and therapy development. Although much progress has been achieved in the respective research fields, to date, it is not possible to fully restore the functions that have been lost or altered due to CNS trauma or neurodegenerative diseases. The development of scaffold materials, which serve as a matrix for transplanted cells, has advanced quite rapidly in the last years. Application of such ECM materials can promote the differentiation of stem cells into neurons and promote additional beneficial effects (Li et al., 2014; Ma et al., 2008; Preston and Sherman, 2011). For cell survival and general cell behavior and function in ECM scaffold-based cell cultures and cell transplantation approaches, it is vital to incorporate additional inducers into otherwise nonbioactive materials (Mammadov et al., 2013). Ideally, for designing a suitable ECM mimetic, it is of great importance to consider both the inhibitory environment, which can result from CNS trauma or inflammation (Fawcett, 2006; Fawcett and Asher, 1999; Fitch and Silver, 2008; Klapka and Muller, 2006), and the different cues in the ECM, which significantly influence the behavior of cells in their surroundings (Chen et al., 2013; Lock et al., 2008; Ulrich et al., 2009). Such cues can be of different chemical, physical, or biological nature, or they may be triggered by cellular interactions or soluble factors. The research of the recent years has brought substantial knowledge regarding these interactions and their underlying mechanisms (Burdick and Vunjak-Novakovic, 2009; Choi et al., 2010; Engler et al., 2009; Kim et al., 2012). Therefore, the design of scaffold materials can—at least in part—be tuned to a specific purpose.

For *in vitro* experiments, to induce neural differentiation, the addition of soluble factors such as chemokines or growth factors to the culture medium is a common and simple approach to mimic one aspect of the interaction of cells with the ECM (Mammadov et al., 2013). The differing responses to various growth factors (neurotrophic factors, e.g., nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3)) have been described for many neuronal subpopulations, and such information is advantageous for culturing the respective cells. As a strategy to mimic the neural ECM, the supply of soluble factors alone has several drawbacks, because it does not take into consideration the three-dimensional network structure of the native ECM, which is important for cellular migration and extension. Matrix materials that resemble the native ECM much more closely

are three-dimensional scaffolding materials that can be modified further, e.g., via combination with the abovementioned soluble factors. Martino et al. (2011) had shown that the immobilization of growth factors on artificial scaffolds has a beneficial effect for tissue regeneration.

A general advantage of hydrogel ECM mimetics is their ability to develop *in situ*. This feature is especially useful when ECM scaffolds are required to bridge tissue defects, which are often irregularly shaped (Estrada et al., 2014; Jain et al., 2006; Sakiyama-Elbert et al., 2012). However, although hydrogels are considered as suitable ECM mimetic substrates whose porosity ensures the supply with nutrients, growth factors, and oxygen to the cell, their porous nature is also disadvantageous. Variation in stiffness causes changes in the porosity that might impair molecular diffusion in the respective scaffold material. Three-dimensional materials can generally provide the structural support, which is necessary for cellular interaction and proper cellular function because it allows the biomolecular cell–matrix interaction.

When designing ECM mimetics, it is important to consider the multiple ECM functions and the complex interaction between ECM components and cells. Model ECMs can be naturally derived or they may be synthetic materials, and they can be biodegradable or nondegradable. Materials from natural sources do have several advantages because of their inherent properties. Although, due to the generally high degree of biocompatibility of natural ECM proteins, the application of these materials appears as a reasonable approach, the results of several recent studies, however, suggest that such approaches may require additional interventions to lead to the desired effects (Banerjee et al., 2009; Engler et al., 2006; Leipzig and Shoichet, 2009). ECM mimetics are usually applied *in vivo* to support multicellular processes, e.g., the formation and regeneration of tissues. Moreover, functionalization of the respective material is often desired, e.g., the addition of bioactive groups such as self-assembling peptides (Maude et al., 2013) or modifications enhancing biodegradability (Liu et al., 2012).

Bioengineered ECM scaffolds can be designed for the controlled release of proteins. In diffusion-based delivery systems, the amount of the released compound can be regulated by physical characteristics, such as the pore size or the degree of porosity, or by the degradation rate of the material (Houweling et al., 1998; Sakiyama-Elbert et al., 2012). Drug delivery can also be achieved by affinity-based systems (Taylor et al., 2006), which allow the controlled release of a substance. An additional possibility is the covalent attachment of the compound to the material (Tian et al., 2005). In this case, the release will occur solely via the degradation of the scaffold material. Such manipulation by modulating physicochemical properties or adding functionalized bioactive groups is a challenge, which is generally more successful in synthetic materials rather than natural matrices.

Furthermore, purification, immunogenicity, and pathogen transmission are important issues that must be considered for ECM-based approaches, and regarding such issues, the design of synthetic materials presents a well-controllable option. Some of the most common natural and synthetic materials that have recently been used as ECM mimetics are described below.

5 BIOMEDICAL NEURAL ECM MIMETICS

5.1 NATURALLY DERIVED ECM MIMETICS

For many *in vitro* cell culture experiments, two-dimensional assays are still the method of choice. However, the generally accepted opinion is that three-dimensional matrices are the preferable ECM model systems because they mimic the physiological situation of *in vivo* tissues more closely (Lutolf and Hubbell, 2005; Mammadov et al., 2013; Owen and Shoichet, 2010). Examples for ECM models of natural sources are matrices such as Matrigel™ (a basement membrane preparation derived from mouse tumor tissue), matrices that consist of individual ECM components, such as collagen, fibrin, or fibronectin, and fragmented or modified ECM components. Natural ECMs are gels that consist of fibrous and fibrillary proteins within a hydrated network of GAG chains. Their structural architecture and their inherent biophysical properties can have significant effects on cellular functions, e.g., adhesion and migration. Naturally derived neural ECM mimetics have been used in therapeutic strategies mostly either as cell carriers for tissue grafting or as regeneration-promoting matrices that are implanted to bridge tissue defects.

5.1.1 Collagen

Collagen plays a dual role for neural growth and regeneration *in vivo*. It is the most abundant protein of native ECMs, and its suitability as a growth-promoting scaffold material has been studied extensively. Collagen's inherent integrin binding sites are advantageous as they promote the migration and differentiation of neuronal cells (Bradshaw et al., 1995). Modification of collagen scaffolds via the addition of neurotrophic factors (Han et al., 2009; Houweling et al., 1998), other pharmacologically active substances (Bolliet et al., 2008), or cells (Joosten et al., 2004) has been described to increase axonal regeneration and functional recovery after spinal cord trauma. Despite the fact that collagen per se is a suitable substrate for neuronal growth, it is also important to consider the role of collagen as the basement membrane constituent of the lesion scar that develops after a traumatic CNS injury (Fawcett et al., 2012; Klapka and Muller, 2006). The collagen matrix of the lesion scar is a sticky matrix to which numerous growth-inhibitory molecules that are upregulated after injury can adhere to and thereby impede neuronal growth.

5.1.2 HA-Derived Materials

HA is a nonimmunogenic linear GAG that is a constitutive of soft connective tissue and is involved in wound healing. It has been reported that HA implantation reduces scar formation in both PNS and CNS (Wang et al., 2012). In this context, its inhibitory effect on the activation of astrocytes, CSPG deposition, and infiltration of macrophages/microglia appears to be dependent on its molecular weight (Campo et al., 2010; Khaing et al., 2011). A disadvantage of HA is that cells do not attach to its surface (Wang et al., 2012). However, its combination with other scaffold materials or functionalization with peptide sequences or other bioactive inducers has been

described to result in suitable mechanical properties for CNS regeneration *in vivo* (Wang and Spector, 2009) and promote neuronal differentiation *in vitro* (Brannvall et al., 2007).

5.1.3 Alginate

Alginate is a water-soluble anionic polysaccharide distributed widely in the cell walls of brown algae, where it forms a viscous gum through binding water. Alginate is a linear copolymer with homopolymeric blocks of (1–4)-linked β-D-mannuronate (M) and its C-5 epimer α-L-guluronate (G) residues, respectively, covalently linked together in different sequences or blocks. Alginate is immunologically inert and is not digested by mammalian cells. As the cross-linking multivalent cations gradually diffuse out from the gel, it slowly degrades and is excreted in urine. Alginate gels are frequently used as matrices for encapsulation of living cells and for the release of proteins (Novikova et al., 2006; Tobias et al., 2001, 2005). In regeneration studies, alginate gels are often applied as cell carrier substances. Alginate has been described to enhance neuronal sprouting and to decrease inhibitory cues after CNS trauma (Kataoka et al., 2004; Prang et al., 2006), but other studies have also reported a lack of axon growth stimulation mediated by alginate scaffolds (Estrada et al., 2014; Novikova et al., 2006). *In* vitro studies have even demonstrated that this biomaterial can inhibit the growth of dorsal root ganglion neurons and cause alteration of the phenotype of various different cell types (Novikova et al., 2006). This gel is, however, a quite suitable matrix for the encapsulation of cells with or even without further modification of the material.

5.1.4 Agarose

The linear polysaccharide agarose is generally well tolerated after its implantation (Tashiro et al., 1997). Although its mechanical properties can be modified to mimic the mechanical properties of its host tissue (Balgude et al., 2001), an agarose ECM mimetic alone—when compared with the effectivity of other natural ECM mimetics—does not suffice to achieve significant degrees of neuronal growth (Lin et al., 2005). Via modification of the material prior to implantation, the neuronal growth rate through agarose scaffolds can, however, be increased (Gros et al., 2010; Jain et al., 2006; Lee et al., 2010; Sakiyama-Elbert et al., 2012; Stokols and Tuszynski, 2006).

5.1.5 Matrigel™

Matrigel™ is a gelatinous ECM protein mixture obtained from the murine Engelbreth–Holm–Swarm sarcoma. It comprises a mixture of Col4, laminin, and heparan sulfate proteoglycan admixed with other minor amounts of extracellular components, as well as growth factors. As a biodegradable implant, Matrigel™ resembles the complex extracellular environment found in many healthy tissues (Kleinman and Martin, 2005) and is widely used as a substrate for cell culture, which forms a nonporous hydrogel at physiological temperature. It has been described as a suitable matrix for tissue generation (Cassell et al., 2001) and has led to neuronal regeneration of varying degrees from only limited growth to extensive axonal sprouting in several studies (Iannotti

et al., 2003; Novikova et al., 2006). Scaffolds of Matrigel™ allow only limited axonal growth after spinal cord trauma (Estrada et al., 2014; Sakiyama-Elbert et al., 2012; Someya et al., 2008). This ECM mimetic, however, seems to exert significant beneficial, growth-promoting effects when it is used as a cell carrier medium in cell transplantation studies. In combination with cellular transplants, especially Schwann cells, Matrigel™ is a widely used biomaterial for neuronal regeneration, where it has been shown to obtain positive results regarding axonal regeneration after central and peripheral nerve injury (Fouad et al., 2005; Novikova et al., 2006; Rodriguez et al., 2000; Someya et al., 2008; Xu et al., 1995, 1997). In contrast to alginate, Matrigel™ has not been found to alter the cellular morphology of olfactory ensheathing glia, Schwann cells, or bone marrow stem cells, and it has been reported to stimulate the growth of dorsal root ganglia *in vitro* (Novikova et al., 2006). Despite reported beneficial effects, the clinical use of Matrigel™ remains questionable because of its tumor-derived origin and its batch-to-batch variability (Sakiyama-Elbert et al., 2012). Batch variation, which might significantly influence the effectiveness, is, in fact, a common disadvantage of natural biomaterials when applied as ECM scaffolds (Orive et al., 2006; Owen and Shoichet, 2010; Wang et al., 2012).

5.1.6 Chitosan

The biodegradable and nontoxic chitosan can be modified to form scaffolds, which contain pores or channels that facilitate neuronal growth (Li et al., 2009). Chitosan is produced via deacetylation of chitin. The manipulation of chitosan and the resulting degree of deacetylation have been reported to influence the adhesion and migration behavior of cells on the surface of the matrix (Chatelet et al., 2001). However, the growth-promoting effects of chitosan scaffolds can be further enhanced via the introduction of functionalized groups or combination with growth factors (Chen et al., 2011; Cheng et al., 2007; Goraltchouk et al., 2006; Nomura et al., 2008; Yu et al., 2007).

5.1.7 Fibrin

The fibrous protein fibrin is involved in the clotting of blood during wound healing. During this process of tissue repair, fibrin molecules form an ECM meshwork, which stimulates cellular proliferation and migration. Two RGD recognition sequences provide binding sites for integrin receptors, which mediate cell adhesion and can significantly influence cellular migration or growth processes (Mosesson, 2005). The use of fibrin scaffolds is safe, lacking side effects. And manipulation of fibrin to create delivery systems that can be used for controlled release of bioactive factors has been tested extensively and yielded positive effects (Johnson et al., 2010; Sakiyama-Elbert et al., 2012; Taylor et al., 2006).

5.1.8 Fibronectin

Fibronectin is a key effector of fibrosis. As a globular plasma protein, it exhibits its soluble form, and as an ECM protein, it takes on a fibrous insoluble form. Via its binding to integrins through specific peptide sequences, it is strongly involved in adhesion-mediated cellular migration (Iannotti et al., 2003). After CNS trauma,

fibronectin ECM mimetics exert growth-promoting and guiding effects on spinal axons (King et al., 2003; Phillips et al., 2004). Additionally, the promotion of cellular infiltration of fibronectin scaffolds with regeneration-supporting glial cells has been described (King et al., 2006).

5.2 SYNTHETIC ECM MIMETICS

Synthetic scaffold materials are generally developed with the intention to fabricate matrices with structural characteristics that resemble those of the native ECM. Although two-dimensional fibrous or fibrillary substrates may result in deviant cellular shapes and behavior, they have proven quite successful in neural tissue engineering approaches where they have been demonstrated to facilitate CNS regeneration (Lutolf and Hubbell, 2005).

The design of synthetic ECMs offers several advantages: bioengineering technologies allow the fabrication of fibrillar biomaterials of nanometer-scale dimensions via polymer processing or supramolecular self-assembly. The typical hydrogel character of native ECM can be physicochemically mimicked by synthetic hydrogels. Synthetic hydrogels can often be formed *in situ*, which makes their application especially attractive for cell-containing hydrogels, which require gentle experimental protocols (Lutolf and Hubbell, 2005).

The possibility to incorporate bioactive signaling cues in both fibrillary- and hydrogel-based neural ECM renders them to be very useful scaffold materials for tissue regeneration.

Both biodegradable and nondegradable synthetic matrices can be fabricated, and their application has been investigated extensively.

5.2.1 Polyethylene Glycol

Polyethylene glycol (PEG) is a fusogen agent, which has been demonstrated to reunite transected cell processes and seal cell membranes after mechanical spinal insult (Borgens, 2001; Borgens and Bohnert, 2001; Borgens and Shi, 2000; Borgens et al., 2002). Acute PEG treatment after spinal cord trauma has also been reported to exert neuroprotective effects via the reduction of oxidative stress reactions (Luo and Shi, 2004; Luo et al., 2004). PEG is a widely used material for the design of biodegradable synthetic cross-linked hydrogels (Burdick et al., 2006; Cong et al., 2009; Gunn et al., 2005; Herten et al., 2009; Phelps et al., 2010; Qiao et al., 2005; Raeber et al., 2005; Rooney et al., 2011). PEG has several beneficial features, which are advantageous for soft tissue regeneration. PEG hydrogels are hydrophilic polymers that generally contain cross-links that exert high degrees of swelling in aqueous environments such as soft tissues (Hoffman, 2002; Lee and Mooney, 2001). PEG is biocompatible and PEG hydrogels are generally not prone to nonspecific cell adhesion or protein adsorption. Such features provide an ideal basis for the introduction of ECM-derived signals, which can stimulate the regeneration of tissues and cells (Chung et al., 2008). Although PEG itself is a material that cells generally do not attach to (Cong et al., 2009), bioengineered PEG hydrogels can also be used as a matrix for the delivery of cells.

Although the most common approaches using PEG for ECM mimetics involve the bioengineering of PEG-containing hydrogels, the generally inert, nontoxic, and nonimmunogenic nature of PEG can also be made use of in a PEG polymer matrix. Beneficial effects of a PEG biopolymer matrix have been described recently. In the respective study, a solution of pure PEG 600 was applied after resection of spinal scar tissue in rats with severe chronic spinal cord injury (Estrada et al., 2014). Although PEG polymers per se do not conform to the basic requirement of an ECM mimetic, which is to provide structural support, they nevertheless promote the formation of a beneficial stable microenvironment *in vivo*. The PEG application promoted the infiltration with cells, which are beneficial for the regeneration of tissue in general and axons in particular. At the same time, the degree of reformation of scar tissue was decreased, which allowed the development of a stable biomatrix at the site of resection. Revascularization, invasion of astrocytes and Schwann cells, and the formation of a stable ECM-containing biopolymer were reported. Furthermore, such PEG treatment alone was sufficient to result in the myelination of regenerated axons and significant locomotor functional improvements in the treated animals (Estrada et al., 2014). Since the chemical properties of PEGs with different molecular weights are nearly identical, the positive treatment effects in the latter study were attributed to the physical properties of the applied PEG (PEG 600): while the tested lower-molecular-weight PEG was too fluid to allow the material to remain at the resection site, the tested PEG of a higher molecular weight even leads to an encapsulation of the implanted material. Only PEG 600—a PEG with a low viscosity—promoted the soft tissue regeneration in the treated area that resulted in the observed cellular invasion and axonal elongation. Such findings argue for the importance of the appropriate stiffness/viscosity that needs to be considered for a respective application.

5.2.2 Lactide- and Glycolide-Derived Polyesters

Poly(lactic acid), poly(glycolic acid), and their copolymers poly(lactic-co-glycolic acid) are widely used biomaterials for tissue engineering strategies. They are biocompatible and biodegradable, and their mechanophysical properties (and thus the rate and timing of degradation) can be modulated to some degree (Sakiyama-Elbert et al., 2012). However, several studies have reported undesired effects or a lack of success that might be explained by the rate of degradation and a loss of structural support (Deumens et al., 2006; Hurtado et al., 2006).

5.2.3 Polycaprolactones

Polycaprolactones are biodegradable polymers, which can be used to fabricate three-dimensional tubular structures with varying degrees of porosity. Implantation of combinations of polycaprolactones with other polysaccharide-based hydrogel materials has been described to reduce inflammation responses after spinal cord injury (Silva et al., 2010). Poly-ε-caprolactone can be used for three-dimensional bioprinting, which makes it a very promising material for novel personalized medical approaches (Wong et al., 2008).

5.2.4 Poly(2-hydroxyethyl Methacrylate)

Poly(2-hydroxyethyl methacrylate) is a nontoxic, nonbiodegradable hydrogel. The material allows the attachment and growth of cells and the transport of small molecules. It can also serve as a guidance substrate for neurons as has been shown for spinal cord trauma (Hejcl et al., 2008). The nonbiodegradable nature can be considered as both advantageous and unfavorable: It ensures the maintenance of a stable scaffold structure over time. At the same time, especially in regard to its clinical application, the scaffold will remain in the patient's body after the implantation. As a foreign substance, it might cause side effects or unpredictable long-term consequences.

5.2.5 NeuroGel™

NeuroGel™ (*N*-(2-hydroxypropyl)methacrylamide or HPMA) is another nonbiodegradable hydrogel that has been described to support cellular infiltration, angiogenesis, and axonal growth after CNS trauma (Woerly, 2000; Woerly et al., 2001). The surface of this scaffold can also be chemically modified via incorporation of bioactive sequences to increase its beneficial effects.

5.2.6 Nanostructured Materials

Nanotechnology offers an enormous potential for the fabrication of nanostructures (particles and fibers). Via electrospinning, nanofiber scaffolds that can be used for cell culturing can be fabricated. Electrospun polyurethane scaffolds with high porosity were previously shown to induce differentiation of hESCs into neurons (Carlberg et al., 2009). In another study, PC12 cells seeded on NGF encapsulated electrospun copolymer of ε-caprolactone and ethyl ethylene phosphate scaffolds were observed to exhibit enhanced neurite outgrowth (Chew et al., 2005). Chemical conjugation to electrospun nanofibers is also effective in inducing neural differentiation. NGF-conjugated aligned electrospun PEG-poly(ε-caprolactone) nanofibers induced transdifferentiation of MSCs into neural cells after 7 days (Cho et al., 2010).

5.2.7 Self-assembling Materials

Self-assembling nanofibers can be tuned in such a way that they form scaffold structures when injected into neural tissue (Berns et al., 2014). Self-assembling scaffolds can be functionalized by using peptide sequences from ECM proteins or other neural differentiation inducers that bind to cell surface receptors. Peptide amphiphiles that self-assemble into nanofibers have successfully been used for neural differentiation *in vitro* and for *in vivo* bridging of CNS tissue defects in several studies (Mammadov et al., 2012a,b; Silva et al., 2004; Tysseling et al., 2010; Tysseling-Mattiace et al., 2008). Self-assembled peptide nanofibers produced from alternating basic, hydrophobic, and acidic amino acids (RADA16) have also been shown to enhance neural cell culture and provide therapeutic effects in several CNS dysfunctions (Ellis-Behnke et al., 2006; Gelain et al., 2011; Guo et al., 2009; Holmes et al., 2000; Silva, 2005). Composites of peptides and nanofibers are very promising scaffold materials for neural growth and regeneration. The incorporation of self-assembling

nanofibers into other matrices makes them very suitable also for cell transplantation-based approaches (Romano et al., 2011).

Generally, nerve grafts—the conventional but limited standard to treat neural defects—have been replaced by the development of novel synthetic scaffolds. The selected list of biomaterials described here covers only a portion of the numerous potential ECM mimetic scaffolds. Recent advances in tissue engineering and nanotechnology demonstrate that neural replacement and repair can be achieved to a remarkable extent. The intensive research regarding development and application of neural ECM mimetics shows that a major determinant is the regulation of cellular behavior by mimicking native ECM features. Desired material properties of neural ECM are mechanical stability, biocompatibility with low or even absence of immunogenicity, controlled biodegradability, and a structure that allows cell migration (porosity) and vascularization. Current technological and engineering methods allow highly specialized designs, which most likely will lead to rapid advances in regenerative medicine in the future.

6 LOST IN TRANSLATION

The replication of the native neural ECM environment is a major challenge. The quest for suitable scaffold materials has led to a better understanding of ECM-mediated functions and has encouraged the development and optimization of suitable bioengineering technologies. The ECM mimetic materials mentioned above only represent a selection of the multitude of biomaterials, which can be used as neural ECM scaffolds. A suitable ECM mimetic needs to exert the principal functions of the native ECM: providing a structural scaffold and mediating cell proliferation, migration, differentiation, survival, regeneration, angiogenesis, and invasion. The orchestration of these cellular processes requires a functional network of biochemical and structural cues.

An important criterion for the design and application of a biomimetic ECM is its translational potential. Regarding the translation from *in vitro* experiments to the *in vivo* development of therapies in large animal models and into clinical applications, it is necessary to prove the effectiveness of a material and its suitability for the intended purpose both *in vitro* and *in vivo*. Moreover and very importantly, safety of the material needs to be demonstrated prior to its application into patients.

Due to the number and complexity of physiological functions, which are mediated by the native ECM, a single formulation cannot meet the requirements for every task (Prestwich, 2008), from cell expansion and delivery to providing a stable scaffold structure and from cell replacement strategies to large-scale repair of tissue defects and regeneration-promoting therapies. For a translation from *in vitro* experiments to clinical applications, it is, therefore, important to optimize the design of ECM mimetic materials with high degrees of flexibility, which would allow the use of few suitable materials for multiple purposes (Prestwich, 2008).

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