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The Role of Extracellular Matrix in Tissue Regeneration

Dwi Liliek Kusindarta and Hevi Wihadmadyatami

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Abstract

Extracellular matrix (ECM) is an extensive molecule network composed of three major components: protein, glycosaminoglycan, and glycoconjugate. ECM components, as well as cell adhesion receptors, interact with each other forming a complex network into which cells reside in all tissues and organs. Cell surface receptors transduce signals into cells from ECM, which regulate diverse cellular functions, such as survival, growth, proliferation, migration, differentiation, and some vital role in maintaining cells homeostasis. This chapter emphasizes the complex of ECM structure to provide a better understanding of its dynamic structural and functional characterization and multipotency. In this chapter the implications of ECM in tissue remodeling are mainly discuss on the neuronal regeneration and wound healing mechanism in the presence of human umbilical mesenchymal conditioned medium (HU-MSCM).

Keywords: extracellular matrix, ECM components, HU-MSCM, wound healing, neuron regeneration

1. Introduction

ECM is a non-cellular structure that regulates almost all of the cellular functions. ECM is a highly dynamic structural network that continuously undergoes remodeling mediated by several matrix-degrading enzymes during normal and pathological conditions. Deregulation of ECM composition and structure has an association with the development and progression of several physiological and pathologic conditions. In this chapter, we describe the structure and function of ECM, also the role of ECM on the wound healing mechanism and neuronal regeneration in the central nervous system (CNS) and peripheral nervous system (PNS).

2. The structure and function of extracellular matrix

An essential part of the holding capacity of tissues is the extracellular area. The extracellular region is primarily occupied by a complicated network of macromolecules constituent called as extracellular matrix (ECM). The composition of ECM is varied, depends on the species and also developing or ground molecules (**Figures 1** and **2**). Commonly, the ECM is composed of three major classes of biomolecules; there are glycosaminoglycans (GAGs), linked to a protein known as the proteoglycans, and also fibrous proteins, including collagen, elastin, fibronectin, vitronectin, and laminin.

In addition, connective tissue (**Figure 3**) is also composed of the matrix of ECM. One of the essential components of connective tissue is fibroblasts and ground substance. Ground substance is a mixing complex between GAGs, proteoglycans, and glycoproteins (mainly laminin and fibronectin). In most connective tissues, the matrix constituents are secreted by fibroblasts, but in several certain specialized types of connective tissues, like cartilage and bone, these components are secreted by chondroblasts and osteoblasts (**Table 1**).

In general, all the cells need to attach to the extracellular matrix to grow and multiply. Extracellular matrix provides support and anchorage for the shape of the cells, regulates and determines cells dynamic and behavior including cell survival, cell proliferation, cell polarity, cell differentiation, cell adhesion, and cell migration. Moreover, the ECM, also gives the mechanical support for tissues and is involved in the growth mechanism, regenerative, and healing processes.

2.1. Glycosaminoglycan (GAGs)

GAGs are unbranched chains of polysaccharides; GAGs are composed of repeating disaccharide units and are heterogeneous groups in negatively charged polysaccharide chains that are covalently linked to proteins to form proteoglycan molecules. The name GAGs is because in

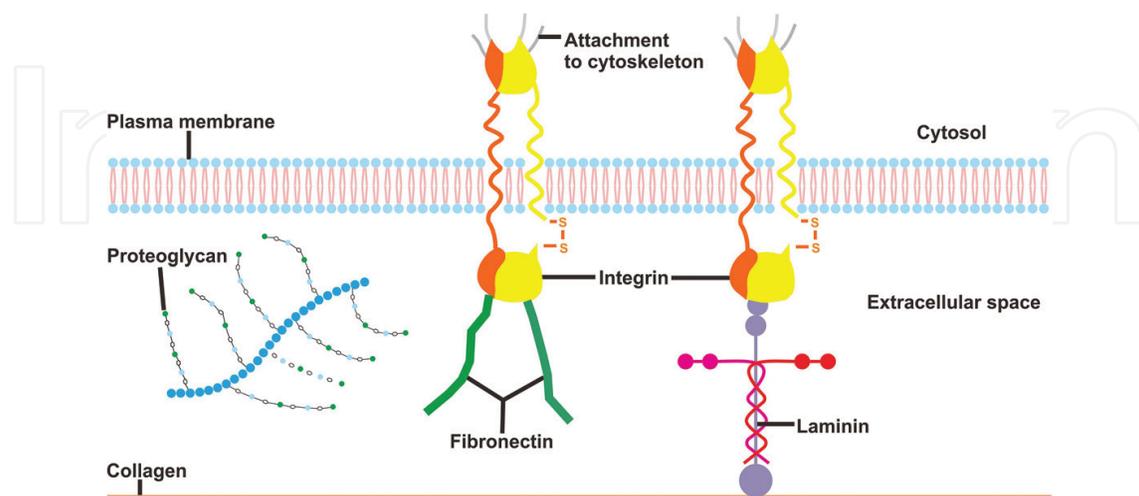


Figure 1. The structure of the extracellular matrix. The ECM mainly contained collagen fibers. There are also some glycoproteins as an adhesion molecule, such as integrin family fibronectin and laminin, which conduct cell attachments to the ECM by binding to collagen in the ECM and integrin. The intracellular part of integrin highly associated with the cytoskeleton thus may promote to anchoring the cell. In the end, there are various proteoglycans in the ECM that act as primary proteins and are profoundly modified by the addition of sugars.

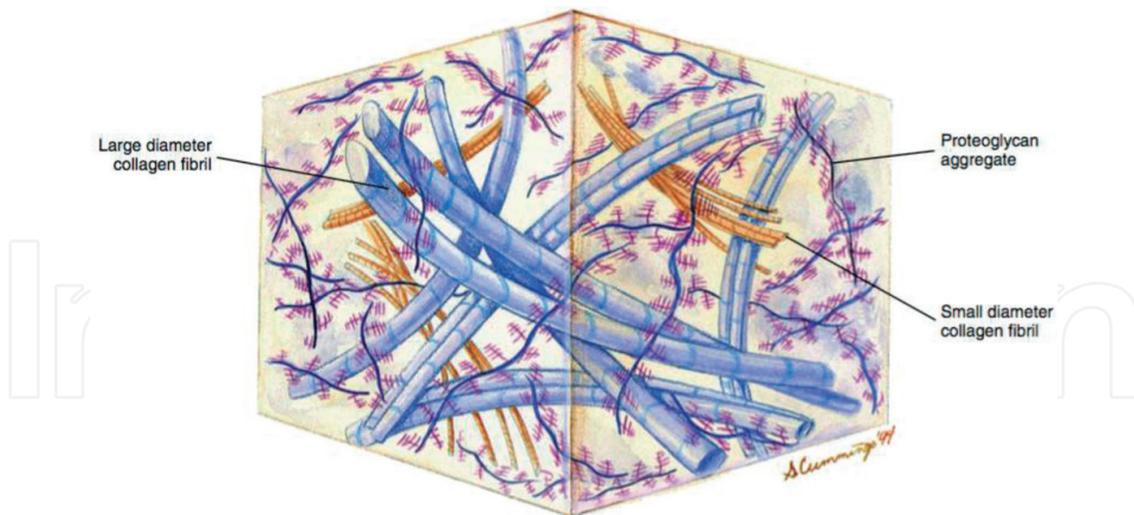


Figure 2. The extracellular matrix of hyaline cartilage found in abundant collagen fibril and proteoglycan aggregates. The chemical analysis of the ground substance reveals that it contains a few glycoproteins and a high concentration of three types of glycosaminoglycans: hyaluronic acid, chondroitin sulfate, and keratan sulfate. Adapted from Crammer and Bakkum [1].

this polysaccharide, one of the two sugars in a repetitive disaccharide is always an amino sugar such as N-acetylglucosamine or N-acetylgalactosamine [3]. The second sugar of GAGs usually is the uronic acid like glucuronic or iduronate. GAG molecules are negatively charged, because there are sulfate or carboxyl groups in most of the sugar. The five main groups of GAGs are differentiated based on the sugar type including (1) hyaluronan or hyaluronic acid, (2) chondroitin sulfate, (3) dermatan sulfate, (4) heparan sulfate, and (5) keratin sulfate. Hyaluronan is the simplest GAGs. Hyaluronan does not contain sulfate sugars; all disaccharides units are the same, and the chain length is extensively big (thousands of sugar monomers). The hyaluronan

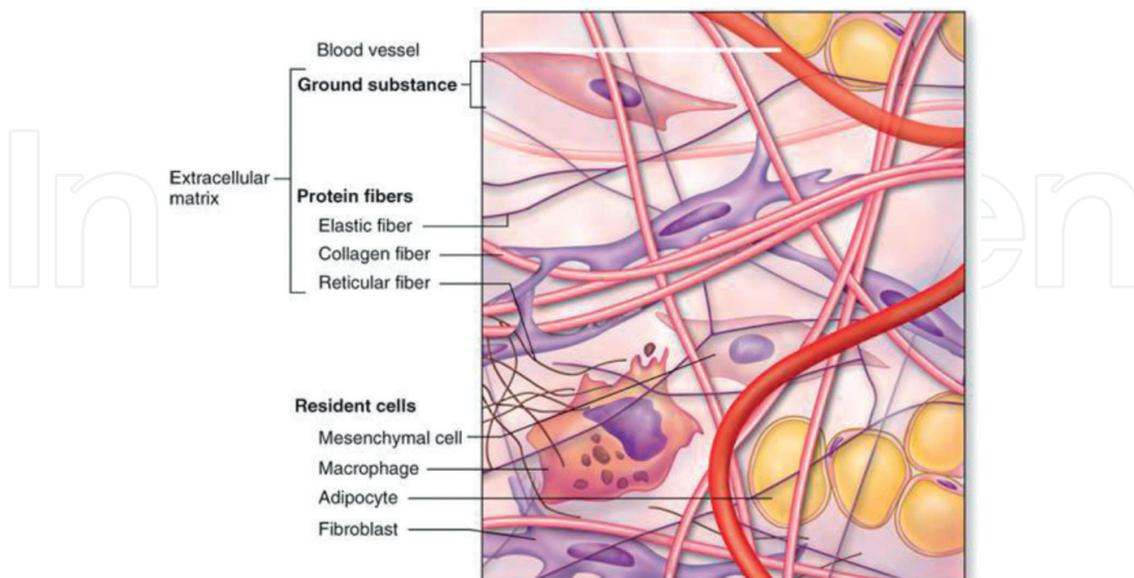


Figure 3. The components of connective tissue. In addition to the extracellular matrix, connective tissues are characterized by a lot of cells, mainly the fibroblasts, all of which are surrounded by the ground substance. Modified from Mescher [2].
Source: Michael W. King; Integrative Medical Biochemistry Examination and Board Review, www.accesspharmacy.com
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Cell Type	Function	Distribution	Characteristics
Fibroblast	Synthesize and secrete collagen, elastic fibers, reticular fiber, and proteoglycan (among other molecules) Support ligaments, tendons, bone, skin, blood vessels, and basement membranes	Throughout all loose and dense connective tissue	Flat, stellate cells with dark, ovoid, staining nuclei, and one or more nucleoli Microscopically may appear to be of different shapes because of the plane of sectioning
Chondroblast	Synthesize and secrete extracellular matrix of cartilage (collagen, elastic fiber and glycosaminoglycans) Support articular cartilage	Present in hyaline cartilage of articulations and fibrocartilage of intravertebral discs Found also in elastic cartilage	Metabolically active with large vesicular nuclei and prominent nucleoli Cytoplasm pale and vacuolated because of high content of lipid and glycogen
Osteoblast	Synthesize and secrete extracellular matrix of bone	In bone	Basophilic cytoplasm resulting from presence of a large amount of rough endoplasmic reticulum that produce glycosaminoglycan and glycoprotein
Myofibroblast	Synthesize and secrete components of extracellular matrix Capable of contractility	In blood vessels and skin throughout the body	Resemble fibroblast under light microscopy but ultrastructurally contain actin filaments for contraction

Modified from Crammer and Bakkum [1].

Table 1. The cells of connective tissue.

is not connected covalently to some core proteins. Proteoglycans are composed of GAG chains that are covalently linked to the core protein and considered to have a significant role in chemical signaling among cells (**Figure 4**).

2.2. Collagen

Collagen is a major abundant fibrous protein in the extracellular matrix. Collagens, which constitute the primary structural element of the ECM, provide tensile strength, regulate cell adhesion, support chemotaxis and migration, and direct tissue development [4]. Recently, there have been already described 28 types of collagen. The main types of collagen found in connective tissues are types I, II, III, V, and XI.

Collagen polypeptide chains are synthesized on membrane-bound ribosomes and fed into the lumen of the endoplasmic reticulum as large precursors, called the pro- α chains. Each pro- α chain then joins the other two to form a hydrogen-bond, triple-stranded hydrogen molecule known as a procollagen. After secretion, the fibrillar procollagen molecule divides to become collagen molecules, which converge into fibrils [5].

2.3. Fibronectin

Fibronectin is an extracellular protein that makes cells adhere to the matrix. Fibronectin is considered as a large glycoprotein found in all vertebrates. Fibronectin usually exists as a

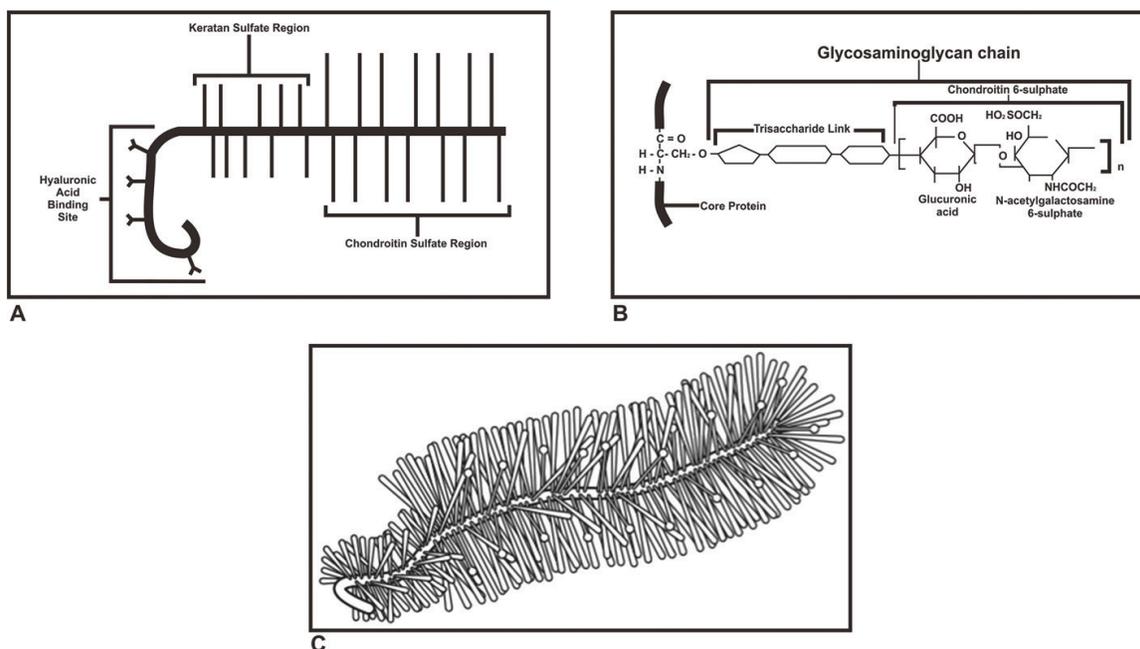


Figure 4. The structure of glycosaminoglycan (A) structure of a proteoglycan monomer. Several glycosaminoglycan chains (chondroitin sulphate and keratan sulfate) attached to a core protein. The protein molecule can connect to a long hyaluronic acid molecule to help form a proteoglycan aggregate. (B) An example of an individual glycosaminoglycan chain, in this case, chondroitin 6-sulphate, and its attachment to the core protein. (C) The morphological of a proteoglycan monomer. (A and B) Adapted from courtesy Dino Juarez, National University of health sciences; (C) Adapted from Crammer and Bakkum [1].

dimer composed of two nearly identical ~250 kDa subunits linked covalently near the C-terminal by a pair of disulfide bonds at one end side. Fibronectin is a ligand member of the integrin receptor family. Integrins are structurally and functionally related to the cell surface as heterodimeric receptors that link the ECM with the intracellular cytoskeleton.

The primary type of fibronectin is known as type III fibronectin replica (cylinder), which binds to integrins. This model has a length of about 90 amino acids. Fibronectin appears in a soluble and fibrillar form. There are two others fibronectin isoforms, which are fibronectin type I (hexagon) and fibronectin type II (square) [6]. Fibronectin is not only crucial for attaching cells to matrices but also to guiding cell migration in vertebrate embryos. Fibronectin has many functions, which allow it to interact with many extracellular substances, such as collagen, fibrin and heparin, and with specific membrane receptors in responsive cells.

3. Tissue regeneration

Extracellular matrix is the primary factor required in the process of forming a new network and tissue. Along with the development found, many different factors can trigger the growth of ECM or used to create a synthetic ECM. Currently, ECM is involved in various mechanisms such as wound healing with or without the involvement of mesenchymal conditioned medium and neuronal regeneration capability associated with pathologic and/or neurodegenerative disease.

The process of wound healing is strongly influenced by the role of migration and proliferation of fibroblasts in the injury site. Indeed fibroblast is one part of ECM. The proliferation of fibroblasts determines the outcome of wound healing. Fibroblasts will produce collagen that will link to the wound, and fibroblasts will also affect the process of reepithelialization that will close the wound. Fibroblasts will produce type III collagen during proliferation and facilitate wound closure. During proliferation stage, fibroblasts proliferation activity is higher due to the presence of TGF-stimulated fibroblasts to secrete bFGF. The higher number of fibroblasts also induces increasing of collagen synthesis. Collagen fiber is the major protein secreted by fibroblast, composed of extracellular matrix to replace wound tissue strength and function. Collagen fibers deposition was significant on 8–10 days after injury. The number of fibroblasts increases significantly, in correlation with the presence of an abundance of bFGF on 8–10 days after wounding.

Mesenchymal stem cell conditioned medium (MSCM) can be defined as secreted factor that referred to as secretome, microvesicle, or exosome without the stem cells which may found in the medium where the stem cells are growing. The use of MSCM as cell-free therapy has more significant advantages in comparison to the use of stem cells, mainly to avoid the need of HLA matching between donor and recipient as a consequence to decrease the chance of transplant rejection. Additionally, MSCM is more easy to produce and save in large quantity. The presence of human umbilical mesenchymal conditioned medium (HU-MSCM), will accelerate curing of the acute and chronic incision and/or burn wound by increasing the number of myofibroblasts and encouraging the expression of VEGF, TGF, bFGF, and also PDGF to promote wound closure.

Recently, it has been mentioned that widespread neuronal cell death in the neocortex and hippocampus is an ineluctable concomitant of brain aging caused by diseases and injuries. However, recent studies suggest that neuron death also occurs in functional aging and it seems in related to an impairment of neocortical and hippocampal functions during aging processes. Data from WHO and Alzheimer report show increasing number of people suffering from dementia along with aging. Profoundly understanding the role of extracellular matrix (ECM) in influencing neurogenesis has presented novel strategies for tissue regeneration (**Figure 5**).

Central nervous system injury because of stroke vascular and amyloid plaque accumulation as the effect of Alzheimer's diseases may cause the disturbance astrocytes, fibroblasts, and oligodendrocyte precursors cell proliferation which may form a glial scar [8, 9]. Within this glial scar, upregulated proteoglycans like CSPGs and changes in sulfation patterns within the ECM result in the building of regeneration inhibition [10].

To solve the problem, some manipulation on the intrinsic extracellular matrix by using traditional herb such as *Ocimum sanctum* extract was already done. In the in vivo and in vitro model using human brain microvascular endothelial cells (HBMECs) which mimics blood-brain barrier, the treatment of the extract may promote the cell proliferation on the hippocampus area and HBMECs in the condition upregulation of choline acetyltransferase (ChAT) enzyme [11, 12]. In addition, there is also a chance to use nanometer-sized scaffolds in the presence of other substrates such as vascular endothelial growth factor or hyaluronic acid with laminin. This scaffold may conduct a way to the regenerative capacity and functional recovery of the CNS to reconstruct formed cavities and reconnect neuronal processes. Thus, the artificial scaffold functions to enhance the communication between cells, allowing for

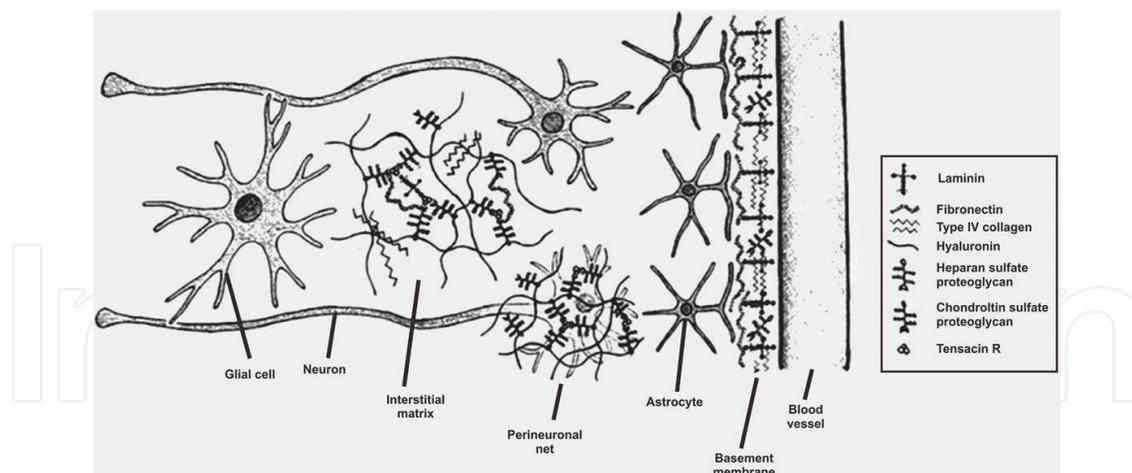


Figure 5. Microscopic anatomy of the extracellular matrix within the central nervous system (CNS). The three major compartments of the extracellular matrix in the CNS are the basement membrane, perineuronal net, and neuronal interstitial matrix. The basement membrane is found surrounding cerebral blood vessels, the perineuronal net is a dense matrix immediately surrounding neuronal cell bodies and dendrites, and the neuronal interstitial matrix occupies the space between neurons and glial cells. Adapted from Lau et al. [7].

improvement in proliferation, migration, and differentiation [13–15]. This evidence gives a new chance in the involvement of HU-MSCM to promote and recover from neuronal injury.

In addition, on the peripheral nerve injury, there is a chance to use scaffold by a chemical decellularization process, acellular nerve allografting that eliminates the antigens responsible for allograft rejection and maintains most of the ECM components, which can effectively guide and enhance nerve regeneration. In the field of tissue engineering by an *in vivo* model, a lot of successful carriers and matrices have been employed as a scaffold to promote direct axonal growth on peripheral nerve injury [16].

In conclusion, the extracellular matrix is the primary factor required in the process of forming a new network and tissue. Along with the development found, many different factors that can trigger the growth of ECM are used to create a synthetic ECM. Recently, ECM is involved in various mechanisms such as wound healing with or without the involvement of mesenchymal conditioned medium and neuronal regeneration capability associated with pathologic and or neurodegenerative disease. In addition, on the peripheral nerve injury, there is a chance to use scaffold by a chemical decellularization process, acellular nerve allografting to eliminate the antigens responsible for allograft rejection and maintain most of the ECM components, which can effectively guide and enhance nerve regeneration. In the field of tissue engineering by an *in vivo* model, significant progress on matrices development have been utilized as a scaffold to promote direct axonal growth on peripheral nerve injury.

Conflict of interest

The authors declare there is no conflict of interest.

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