Review Article
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Laminin Enriched Scaffolds for Tissue Engineering Applications

Abstract

Laminin (LM) is heterotrimeric large molecular weight glycoprotein that forms a crucial component of the basal lamina (or basement membrane) of most tissues. LMs are integral for cell adhesion, proliferation, survival, migration, and differentiation. Several studies have used a LM-coated 2D substrate for the culture and maintenance of stem cell phenotype in vitro. Recent studies have reported that LM can also be incorporated in 3D scaffolds for tissue engineering applications. This article illustrates the bioactivity and regenerative potential of LM protein in 3D scaffolds for skeletal muscle, nerve, vascular, and intervertebral-disc regeneration.

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Muhamed Talovic, Madison Marcinczyk, Natalia Ziemkiewicz, Koyal Garg
Department of Biomedical Engineering, Saint Louis University, USA

Correspondence: Koyal Garg, Assistant Professor, Department of Biomedical Engineering, Parks College of Engineering, Aviation and Technology, Saint Louis University, 3507 Lindell Blvd., St. Louis, MO 63103, USA, Tel 314-977-8200, Email gargk@slu.edu

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Abbreviations: ECM, extracellular matrix; LM, laminin; 3D, three-dimensional; VML, volumetric muscle loss; PDMS, polydimethylsiloxane; PEG, poly ethylene glycol; NMJ, neuromuscular junction; AchR, acetylcholine receptor; PGA, polyglycolic acid; NGF, nerve growth factor; PEGDA, PEG-diacrylate; PLLA, poly l-lactic acid; SVZ, sub ventricular zone; CNS, central nervous system; SGZ, sub granular zone; TBI, traumatic brain injury; NPSC, neural progenitor/stem cells; SDF, stromal cell derived factor; IVD, the intervertebral disc; NP, nucleus pulposus

Introduction

Basement membranes are sheet-like extracellular matrix (ECM) structures that separate epithelium from the underlying connective tissue and surround nerve, muscle, and vessel cells.[1] The basement membrane provides mechanical stability and plays crucial roles in cell differentiation, survival, and migration. Major components of the basement membrane include collagen type IV and a heterotrimeric glycoprotein, laminin (LM).[2] LM is composed of three chains (α, β and γ) that are known to spontaneously self-assemble into hexagonal networks *in vitro* through calcium dependent interactions.[3] LM exists in multiple isoforms in different tissues. Five α chains, three β chains and three γ chains have so far been identified. Combinations of these chains lead to the formation of 16 different LM isoforms with tissue-specific functions. For example, LM-111 ($\alpha_1\beta_1\gamma_1$ or LM-1) is expressed in epithelium and endothelium, LM-211 ($\alpha_2\beta_1\gamma_1$ or LM-2) is present in striated muscle and peripheral nerves, LM-121 ($\alpha_1\beta_2\gamma_1$ or LM-3) exists in synapse and glomerulus, LM-221 ($\alpha_2\beta_2\gamma_1$ or LM-4) is expressed in myotendinous junction, and LM-332 ($\alpha_3\beta_3\gamma_2$ or LM-5) is found in skin and mucous membranes.[3,4] The distribution of LM-111 in adult tissues is limited and it is primarily expressed during fetal and embryonic development.[5] LM has been incorporated in three-dimensional (3D) scaffolds for the regeneration of several different tissues such as skeletal muscle, nerve, intervertebral-disc, and blood vessels. Some recent findings are summarized in this article.

Skeletal muscle regeneration

LM is a structural protein in the ECM of skeletal muscle fibers that provides an important scaffold for tissue development, maintenance, and function.[6] Satellite cells and myoblasts express the $\alpha_7\beta_1$ integrin and interact with LM in the basal lamina. LM influences satellite cell proliferation, adhesion, migration, and differentiation.[7–10] and LM-deficient (dy/dy) mice are reported to lose their endogenous capacity for regeneration.[11–13] LM-111is one of the first proteins expressed during embryogenesis and is associated with a variety of biological activities, including stem cell migration, nerve growth, angiogenesis, matrix remodeling, and basement membrane assembly.[14] LM-111 supplementation has demonstrated remarkable regenerative capacity in several models of muscular disease[15–17] and injury,[18] primarily by enhancing satellite cell activity. In mice with congenital muscular dystrophy due to missing LM α_2 chains, overall health and survival can be improved by administration of LM α1 chains,[19,20] *In vitro*, LM-111 promotes muscle stem cell proliferation and differentiation to a greater extent than other ECM components such as collagen type I and fibronectin.[21] The development of LM enriched 3D scaffolds will be useful for the treatment of large muscle defects in cases of volumetric muscle loss (VML) where long-term structural and mechanical support is required for there generating muscle tissue.[22–25] Given the therapeutic efficacy of LM in skeletal muscle regeneration, studies incorporating LM in tissue engineered 3D scaffolds are critically needed to improve orthopedic care of musculoskeletal trauma.

Dennis and Kosnik engineered 3D skeletal muscle tissue constructs termed myooids by culturing primary skeletal muscle myoblasts on a LM coated (0.5-1.5μg/cm^2) SYLGARD substrate with anchor points composed of decellularized muscle or LM coated silk suture.[26] After 2-3 weeks of culture, the monolayer of myotubes in each dish detached from the SYLGARD substrate and rolled into a cylinder while only remaining attached to the anchor points. Once formed, these myooids produced a twitch force of 215μN, maximum isometric force of 440μN and a specific force of 2.9kN/m^2 in response to electrical stimulation *in vitro*.

Using a similar concept, micro-patterned substrates of polydimethylsiloxane (PDMS) were coated with LM at a density of 2μg/cm^2 to provide an aligned and adhesive substrate for primary myoblast differentiation.[27] LM coated silk sutures were pinned into the PDMS plates to serve as anchor points. Fibrin gel (20mg/ml) was added on top of the confluent myotubes to facilitate cell detachment

from the substrate into a single intact cylindrical layer between the anchor points. The pre-aligned skeletal muscle constructs stained positive for myosin heavy chain and produced significantly higher contractile forces compared to unaligned constructs. The pre-aligned muscle constructs produced a peak twitch force of 215μN, peak tetanus force of 411μN, and specific force of 8.10kN/m^2 *in vitro*.

Bursac and co-workers created bioengineered myobundles composed of fibrinogen (4mg/ml) and matrigel (40%) mixed with neonatal rat derived skeletal muscle myoblasts.[28] The myobundles were held under tension during culture and resulted in the formation of highly aligned and cross-striated myotubes (α7 integrin$^+$ and α-actinin$^+$) that were capable of isometric force production (~3mN). The increase in myofiber hypertrophy and prolongation of intracellular calcium transients in the bioengineered myobundles provided the mechanistic basis for the high levels of contractile force generation *in vitro*. In a subsequent study, Bursac and co-workers modified the biomimetic hydrogels by combining 20mg/ml fibrinogen with ~32% matrigel and human skeletal muscle myoblasts.[29] The myobundles were reported to generate electrically induced calcium transients and tetanic contractions (specific force~7mN/mm^2) as well as force-length and force-frequency relationships, recapitulating key functional aspects of human skeletal muscle. Additionally, the myobundles maintained functional acetylcholine receptors and underwent dose-dependent hypertrophy or toxic myopathy in response to pharmacological treatments *in vitro*.

In another study, LM was covalently cross-linked at a density of 7.6ng/cm^2 to a poly (ethylene glycol) (PEG) hydrogels.[30] Hydrogels that mimicked the native elasticity of skeletal muscle (~12kPa) promoted skeletal muscle derived satellite cell renewal in culture. The study further showed that muscle satellite cells cultured on LM functionalized pliant hydrogels are able to support extensive muscle regeneration when implanted into immune deficient mice depleted of endogenous satellite cells by irradiation. However, the study did not evaluate functional improvements in the skeletal muscle following cell transplantation. Taken together, these studies demonstrate that LM can be blended, coated or covalently cross linked on 3D scaffolds to support functional skeletal muscle regeneration.

Peripheral nervous system regeneration

Besides influencing satellite cell activity, LM-111 is also crucial for maintaining nerve cells in peripheral nerves[31] and serves as a major substrate for neurite extension[32] and axon growth,[33] both *in vivo* and *in vitro*. After peripheral nerve injury, LM production by Schwann cells at the injury site is up regulated to promote axonal regeneration. Mice lacking LM α_2 chain show impaired axon myelination due to reduced Schwann cell proliferation. Similarly, humans suffering from congenital muscular dystrophy due to the lack of LM α_2 develop demyelinating peripheral neuropathy.[34] The absence of LM also results in impaired neuromuscular junction (NMJ) development.[35–37] and exogenous LM-111 supplementation promotes acetylcholine receptor (AChR) clustering and functional NMJ formation *in vitro*.[7,21,38] LM and agrin can act together to enhance the number, size, and rate of AChR clusters.[38,39] LM also mediates the interaction between integrin $\alpha_7\beta_1$ and the neural AChR clusters on the muscle membrane, potentially stabilizing them and allowing them to be more readily innervated by the incoming neurite.[40] As a result, LM based neurogenic materials have been widely used as peripheral nerve grafts in several studies.

A number of studies have combined LM with collagen to create 3D scaffolds for peripheral nerve regeneration (Table 1). Harkins and co-workers studied the impact of LM (0-100μg/ml) on 3D neurite extension in neurons dissociated from E9 chick dorsal root ganglion in collagen (0.4-2.0mg/ml) gels.[41] LM was shown to associate homogenously with collagen fibers and did not alter the mechanical properties of the collagen gels. Neurite outgrowth was greater on gels composed of lower collagen (0.4-0.8mg/ml) and LM (1-10μg/ml) concentrations, indicating a dose-dependent effect of ECM components on neuron differentiation and neurite out growth.

Toba et al.[42] developed a novel nerve conduit composed of polyglycolic acid (PGA)-collagen tube filled with human LM (10g/ml) soaked collagen sponge that was implanted into an 80-mm gap of peroneal nerve in dogs. At 12months post-surgery, the conduit was completely absorbed and a high density of myelinated axons could be observed in the regenerated nerve segment. Electrophysiological studies showed that both motor and sensory nerves had partially regenerated and re-established electrical connections with their target tissues. Since LM is known to promote nerve regeneration directly, the authors hypothesized that local release of LM from the collagen sponge may have accelerated the regeneration of elongating axons that were able to bridge the wide neural defect and form connections with the distal nerve stump while the implanted guiding conduit was still intact.

In a recent study, a scaffold combining collagen with LM was able to promote greater nerve regeneration in a rat model of recurrent laryngeal nerve injury compared to pure collagen scaffold.[43] The functional recovery matched that of autologous nerve grafts when LM was used in combination with collagen. Although motor function was not restored, injuries treated with the collagen tube loaded with LM and also LM binding neurotrophic factors was able to improve vocalization, arytenoid cartilage angles, compound muscle action potentials, and regenerated nerve fiber area in comparison to the autograft treated group.

Suri et al.[44] fabricated hydrogels composed of hyaluronic acid, collagen and LM for the encapsulation of Schwann cells. The encapsulated cells showed higher metabolic activity and proliferation on LM enriched hydrogels. The Schwann cells also secreted highest levels of neurogenic growth factors such as nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) in hydrogels that contained LM. Cells in the LM enriched hydrogels were also found to align parallel to each other and form structures called Bands of Bungner suggesting the formation of oriented channels for axonal guidance and synapse formation. Furthermore, the hydrogel matrix was shown to support the co-culture of Schwann cells and neurons.

LM has also been combined with synthetic polymers such as PEG for peripheral nerve regeneration. PEG-LM111 hydrogels were synthesized by reacting them with PEG-diacrylate (PEGDA) in the presence of irgacure as an initiator.[45] In contrast to findings reported by other studies,[46] the hydrogel stiffness decreased with increasing concentrations of PEG-LM111 conjugates. Hydrogel encapsulated dorsal root ganglion exhibited longer neurite extensions with increasing LM111 concentration (0-100μg/ml). The authors reported no difference between LM111 conjugation and encapsulation within the PEG hydrogels confirming that chemical conjugation had minimal effect on the LM111 protein structure and bioactivity. Interestingly, the study also showed that LM concentration had a greater impact on neurite extensions than the stiffness of the gels.

In addition to hydrogels, LM has also been combined with electrospun nanofibers. LM was combined with poly (l-lactic acid) (PLLA) nanofibers using covalent binding (~1.75μg/mg of PLLA), physical adsorption (~1.50μg/mg of PLLA), and blended electrospinning (~3.12μg/mg of PLLA).[47] LM modified PLLA scaffolds supported better PC12 cell viability and adhesion compared to unmodified nanofibers. Additionally, blended LM-PLLA nanofibers promoted greater neurite outgrowth compared to surface modified nanofibers. Three-dimensional incorporation of LM into PLLA scaffolds resulted in greater overall concentration and better cellular interaction. Taken together, these studies suggest that LM can be combined with a wide range of natural and synthetic materials to support the proliferation and maturation of Schwann cells and neurons for peripheral nerve regeneration.

Table 1 Number of studies have combined LM with collagen to create 3D scaffolds for peripheral nerve regeneration

Scaffold composition	Morphological properties	Mechanical properties	Cell and tissue interactions	Reference
Skeletal muscle regeneration				
PEG functionalized with LM(7.6ng/cm^2)	N/A	Elastic modulus ~12kPa	Hydrogels mimicked native tissue elasticity and enhanced satellite cell renewal *in vitro*. Implantation of hydrogels in satellite cell deficient mice supported extensive muscle fiber regeneration.	67
Fibrinogen (4mg/ml)+Matrigel (40%)	N/A	N/A	Hydrogels supported aligned and cross-striated myofiber formation from encapsulated myoblasts and generated contractile force (~3mN) *in vitro*.	28
Fibrinogen (20mg/ml)+Matrigel(~30%)	N/A	N/A	Hydrogels supported striated myofiber formation from human myoblasts and produced specific force of 7mN/mm^2 for tetanus. Myobundles exhibited physiological responsiveness to pharmaceutical treatment.	29
LM coated (0.5-1.5μg/cm^2) SYLGARD dish with LM coated anchor points	N/A	N/A	Monolayer of rat myoblasts rolled into a cylindrical structure called myooid that produced a specific force of 2.9kN/m^2 in response to electrical stimulation.	26
LM (2μg/cm^2) coated micro patterned PDMS with anchor points and fibrin gel (20mg/ml)	N/A	N/A	Fibrin gels were used to detach a monolayer of aligned myotubes cultured on micro patterned PDMS, which rolled into a cylindrical construct between the anchor points and produced a specific force of 8.10kN/m^2 *in vitro*.	27
CNS regeneration				
LM(10μg/ml) rich gelatin (3%) sponge	N/A	N/A	Transplanted sponge supported neuroblast migration and suppressed microglial invasion into cortical lesions of mice.	50
LM(0.01% w/v) and gelatin (4.4 % w/v) cryogel	Mean pore size of 80-120μm	N/A	Cryogels promoted human cord blood-derived stem cells differentiation into neural tissue-like structures in vitro. Cryogel implantation in rat brain cortex supported neuroblast infiltration.	52
LM-111(~1.6 ng/mg) tethered to methylcellulose to form a hydrogel	N/A	Complex moduli 79-155.9 Pa	Thermo responsive injectable hydrogel enhanced primary rat cortical neuron adhesion and survival.	52
LM-111(0.015% w/v)+hyaluronic acid(1.75% w/v) hydrogels	Pore size ranging from 2-17μm	Storage modulus of 1.02kPa	Hydrogels supported NPSCs viability and up regulated their chemotactic migration in response to SDF-1α *in vitro*.	53
Hyaluronic acid(1 mg/ml)+LM (500 ng/ml)	Pore-size ranging from 6-60μm	Complex modulus ~ 400 Pa	Transplantation of hydrogels in a rat brain lesion showed astrocyte and microglial infiltration, angiogenesis, neurite extension and regeneration of myelinated nerve fibers	68
LM (100μg/ml)+hyaluronic acid (1 mg/ml)+fibrin (5mg/ml) hydrogels	N/A	N/A	Hydrogels enhanced neurite outgrowth from differentiating NPSCs and enhanced angiogenesis from endothelial cells in co-cultures.	55
PNS Regeneration				
LM (10μg/ml)+collagen (0.4-0.8 mg/ml)	N/A	Modulus:10-100dyn/cm2	Hydrogels supported neurite extension from dorsal root ganglion in a dose dependent manner.	41
PGA-collagen tube filled with human LM (10g/ml) soaked collagen sponge	N/A	N/A	Implantation of LM-collagen sponge in dogs enhanced nerve regeneration across 80-mm wide neural defect, with partial motor and sensory function recovery.	43
Collagen tube loaded with LM and LM binding neurotrophic factors	Mean pore size of 60±4 μm.	N/A	Transplantation of scaffold in a rat model of laryngeal nerve injury improved vocalization, arytenoid cartilage angles, compound muscle action potentials and regenerated nerve fiber area.	44
Collagen, Hyaluronic acid and LM (100μg/ml)	N/A	N/A	Encapsulated Schwann cells increased NGF and BDNF secretion in the presence of LM.	45

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Table continued...

Scaffold composition	Morphological properties	Mechanical properties	Cell and tissue interactions	Reference
PEGDA(3%)+PEGLM(100µg/ml)	N/A	Mechanical stiffness, ~70 Pa	Hydrogels supported longest neurite extension from dorsal root ganglion dissociated neurons.	45
LM electrospun with PLLA(3.12µg/mg)	N/A	N/A	LM modified PLLA scaffolds supported greater PC12 cell viability and adhesion and greater neurite extension compared to plain PLLA scaffolds.	69
IVD regeneration				
PEG-octoacrylate and PEG-dithiol + PEGLM111 conjugates (500µg/ml)	N/A	Elastic Modulus, 1200-1500 Pa	Hydrogels significantly improved encapsulated porcine NP cell survival and retention over a 14 day period in rat IVD explants.	58
PEG and LM (2mg/ml) (25: 1 molar ratio)	N/A	Shear modulus, 0.9-1.4kPa	Hydrogels supported primary NP cell survival and GAG synthesis. Encapsulated NP cells showed increased expression of N-cadherin and cytokeratin-8.	45
Vascular regeneration				
Collagen (2mg/ml) + LM-5 (50µg/ml) hydrogels	N/A	N/A	Hydrogels interspersed with HUVECs and HBMSCs supported cell proliferation and formation of tubular network	60
ePTFE functionalized with LM-1 (25µg/cm^2)	N/A	N/A	LM immobilized small diameter ePTFE vascular grafts remained patent and supported neovascularization and endothelialization 5 weeks post-implantation in a rat model.	61

Central nervous system regeneration

Neurogenesis occurs in two specialized niches of the brain which are localized in the sub granular zone (SGZ) of the dentate gyrus in the hippocampus and the sub ventricular zone (SVZ) of the lateral ventricles of the human brain.[48] In both zones, neurogenesis is closely associated with angiogenesis. LM is a major component of the basement membrane of blood vessels in the central nervous system (CNS) and is responsible for regulating neuronal migration and neurite outgrowth. Neural stem cells receive signals from interstitial LM and from the fingerlike processes of the basal lamina called fractones, which extend from blood vessels.[49] These integrin mediated cellular interactions with LM are vital for neuronal proliferation, survival, migration and differentiation.[32]

Lack of effective therapies to restore lost brain neurons following stroke or injury presents a significant opportunity to develop clinically relevant neurogenic materials. Ajioka et al.[50] created LM containing porous gelatin sponges for enhancing neuroblast migration in a rodent model of traumatic brain injury (TBI). LM concentration of 10µg/ml was sufficient to elicit astrocyte and neuroblast migration into the cortical lesions and suppress microglial cell invasion. Moreover, LM enriched sponges were wrapped with astrocytic processes, resembling the structure of blood vessels in the CNS.

Jurga et al.[51] created cryogels containing LM (0.01% w/v) and fish skin gelatin (4.4% w/v) that were crosslinked with glutaraldehyde for mechanical stability. In hippocampal organotypic cultures, the gelatin-LM cryogels showed intensive migration of NF200$^+$ neuroblasts throughout the entire volume of the scaffold. GFAP$^+$ glial cells were found constricted to the periphery of the scaffold and did not form dense scars typical of brain tissue lesion. Additionally, LM-rich cryogels did not promote microglial cell activation and infiltration into the scaffolds. The authors observed a similar integration process following cryogel implantation in the rat brain cortex following TBI. The cryogels also promoted neuronal differentiation of human cord blood derived stem cells and formation of neural niche-like structures *in vitro*.

Stabenfeldt et al.[52] tethered ~1.6ng of LM-111 per mg of methylcellulose to create a thermo responsive hydrogel for neural tissue engineering. The hydrogel was formed without a free radical initiator to avoid potential cytotoxic effects and could be delivered via minimally invasive techniques. Primary rat cortical neurons showed increased adhesion and survival on LM-111 functionalized hydrogels compared to unfunctionalized controls. Overall, the study showed that a bioactive and injectable hydrogel that could gel in situ could offer significant advantages in treating CNS trauma, where an irregularly shaped lesion cavity often poses structural and integration difficulties for pre-formed tissue engineered constructs.

To overcome issues of low survival and viability post-transplantation of neural progenitor/stem cells (NPSC) in traumatic brain injury, Addington et al.[53] synthesized LM immobilized hyaluronic acid (HA) hydrogels for NPSC delivery and engraftment. Hydrogels containing high concentrations of LM (0.015%w/v) and low concentrations of HA (1.75%w/v) mimicked the stiffness of native neural tissue (1.02kPa) and supported greater NPSC density, viability, and chain length. The HA-LM hydrogels up regulated CXCR4 expression in NPSCs after 48 hours of culture and promoted their chemotactic migration in response to stromal cell derived factor (SDF)-1α gradients. Taken together, these results suggest that implantation of a HA-LM hydrogel can enhance the delivery and migration of NPSC in an injured microenvironment.

In another study, LM (500ng/ml) was cross-linked onto the backbone of hyaluronic acid hydrogels. Both LM modified and unmodified hydrogels were evaluated for their ability to repair and regenerate CNS after trauma. 6 and 12 weeks after implantation, both hydrogels were found well integrated with the host tissue. The scaffolds showed astrocyte and microglial infiltration as well as angiogenesis but only LM modified scaffolds showed neurite outgrowth and the presence of myelinated nerve fibers in the regenerating tissue.[54]

Arulmoli et al.[55] combined fibrin, HA and LM (100µg/ml) to create combination scaffolds for the purpose of NPSC transplantation following CNS injury. The addition of LM significantly improved neurite outgrowth from differentiating NPSCs. The presence of LM

and NPSCs in the scaffolds synergistically enhanced angiogenesis from endothelial cells. Co-culture of NPSCs with human cord blood-derived endothelial cells on the composite scaffolds containing LM yielded the highest vessel area percentage, total vessel length, and significantly more branch points.

Intervertebral disc regeneration

The intervertebral disc (IVD) is composed of a thick outer ring of fibrous cartilage termed the annulus fibrosus, which encapsulates a gelatinous core known as the nucleus pulposus (NP). The NP contains collagen and elastin fibers embedded in an aggrecan-containing gel.[56] Age associated degeneration of IVD originates in the NP region and is characterized by decreased water-content, cellularity as well as proteoglycan and ECM composition resulting in impaired ability of the tissue to resist and redistribute compressive loads.[57] NP cells are known to cluster and produce more proteoglycan rich matrix when cultured on soft (<0.5kPa) LM rich substrates as compared to fibronectin or collagen.[57] Francisco et al.[58] developed injectable, LM-111 functionalized PEG hydrogels by combining PEG-LM111 conjugates with PEG-octoacrylate and PEG-dithiol under physiological conditions. Bioluminescence studies showed that PEG-LM111 hydrogels significantly improved encapsulated NP cell survival and retention over a 14 day period in IVD explants.

In a subsequent study, Fransisco et al.[46] synthesized photocrosslinkablePEG-LM111 hydrogels for IVD regeneration. Increasing the degree of LM modification was found to alter LM bioactivity and inhibit immature NP cell adhesion. The stiffness of the hydrogels was shown to increase with increasing concentrations of PEG-LM111 conjugates. Primary NP cells formed large multi cell clusters and increased glycosaminoglycan (GAG) synthesis when cultured on soft PEG-LM111 hydrogels. Cell encapsulation in 3D PEG-LM111 hydrogels was found to influence NP metabolism and stimulated higher expression of N-cadherin and cytokeratin 8. These studies provide evidence that LM111 enriched soft substrates can positively influence NP cell proliferation and function for treating age-associated IVD degeneration.

Vascular regeneration

Rapid vascularization of tissue engineered grafts is important for regeneration. Restoration of blood flow in peripheral vascular disease and stroke is crucial for the survival of ischemic tissues. LM is a major biologically active component of the vascular basement membrane that promotes endothelial cell adhesion, migration, and differentiation to support angiogenesis.[59]

Stamati et al.[60] fabricated type 1 collagen hydrogels supplemented with of 50µg/ml of LM-5. The hydrogels were interspersed with human umbilical vein endothelial cells (HUVECs) and bone marrow derived mesenchymal stem cells (HBMSCs) throughout the matrix to recapitulate the *in vivo* 3D microenvironment of a vascular niche. While collagen hydrogels supported cobblestone morphology in HUVECs, the addition of LM resulted in HUVEC aggregation and formation of large end-to-end tubular networks. The authors attributed the formation of these tubular structures to the increased expression of α_6 integrin and vascular endothelial growth factor receptor 2(VEGFR2) in HUVECs as well as enhanced VEGF uptake by the cells in the presence of LM-5.

Surface modification with LM is also reported to enhance anticoagulant and endothelialization properties of vascular grafts. LM-111 was covalently immobilized on the surface of synthetic polymeric vascular grafts such as expanded poly tetra fluoroethylene (ePTFE).[61] These LM modified small diameter vascular grafts were tested as interpositional aortic grafts in a rat model. At 5 weeks post-implantation, the LM modified grafts showed increased neovascularization and endothelialization compared to unmodified ePTFE. LM modified grafts also remained anti thrombogenic and patent and did not show evidence of intimal thickening, overcoming the disadvantages commonly associated with small diameter synthetic vascular grafts. In another example, co-immobilization of LM and fucoidan, a sulfated polysaccharide, on a glass substrate improved endothelial cell proliferation but inhibited smooth muscle cell growth and platelet adhesion.[62] Taken together, these studies suggest that surface modification with LM can enhance the anticoagulant properties and endothelialization of vascular grafts.

Conclusion

LM has been successfully combined with several synthetic and natural polymers in 3D scaffolds to promote tissue regeneration. The studies reviewed in this article illustrate that LM concentration and method of incorporation into the 3D scaffold can greatly influence its bioactivity and capacity for regeneration. A number of studies have used short peptide sequences from LM chains to promote cellular activity in 3D scaffolds.[63–66] While these short peptides are more stable and easily synthesized, they do not recapitulate the 3D microenvironment of the stem cell niche in adult tissues. Matrigel is often used in tissue engineered scaffolds as it contains high concentration of LM. However, the concentration of LM in Matrigel is variable and cannot be precisely controlled for dose-dependent effects. Additionally, both Matrigel and LM are commonly isolated and purified from rodent tissue which is a major impediment to their clinical translation. Thorough evaluation of the regenerative potential of full-length human LM enriched scaffolds in large animal models will pave the way for clinical translation of this therapeutic protein.

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Conflict of interest

The author declares no conflict of interest.

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