STEM CELL CULTURE MATRICES
A practical guide

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To develop a differentiation protocol that generates the target cell type at a sufficient quantity and purity and with high phenotypic maturity is challenging. Following this, the advancement from pre-clinical studies to a Phase 1 clinical trial requires a well-controlled production process and a safe and efficacious cell product.

Lessons from developmental biology have resulted in many protocols that successfully recapitulate the temporal developmental processes via well designed medium formulations. Now, slowly, the important role of the extra-cellular matrix in shaping the cell culture microenvironment is also getting its rightful recognition, where adhesion is not only about making cells stick, but also about the interaction between the culture substrate and cells which can influence cell behavior.

Over the past decades, a stride of different reagents for stem cell maintenance and differentiation has been commercialized. Selecting the right substrate is often a painstaking and time-consuming task, because of the wide variety of products on the market, the lack of transparency when it comes to various components, and the large number of variables to consider for each application. This guide was created to help demystify the choices and simplify the selection process.

1. Matrices and Stem Cell Culture

Rapid advances in stem cell technology are unlocking exciting new opportunities for biomedical research, drug discovery and cell therapy. Big breakthroughs over the past decade have improved our understanding of stem cell biology immensely. Nevertheless, robust and reproducible stem cell culture and differentiation systems are still notoriously difficult to establish and validate, especially for demanding applications such as screening, commercial production and highly regulated clinical research purposes.

Stem cell methodologies vary widely depending on the end goal, but one thing is common to all of them: successful results depend critically on how closely the culture system mimics the stem cell's natural environment. In particular, the culture substrate, or matrix, on which stem cells are grown can dramatically alter their phenotype, behavior and fate. Surprisingly, however, the importance of the stem cell culture matrix is frequently under-appreciated or overlooked completely during system development and even process optimization. The use of biologically irrelevant and poorly defined matrices is still commonplace.

The type of cell culture substrate used will have a great impact on your research. Selection of an inappropriate substrate can compromise validity of your culture system, increase the risk of contamination, and introduce unnecessary variability - all of which raise your chances of poor quality results, unforeseen set-backs, and costly failures. Yet, scientists still use undefined cell culture substrates where many of them are facing issues, such as low cell yield and bad cell quality, and are struggling with laborious, inconsistent and operator-dependent procedures. Moreover, the majority of the scientist state that they have issues maintaining a homogenous stem cell population, having to spend time on manually removing differentiated cell areas (Figure 1).

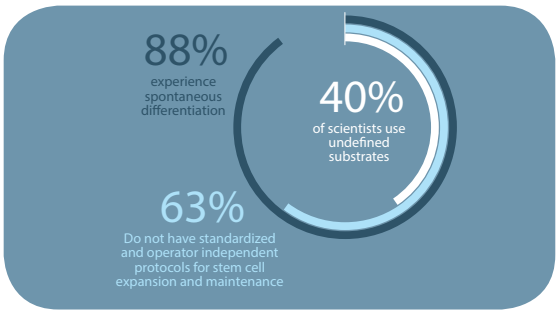

Figure 1. The type of cell culture substrate used will have a great impact on the research results and will affect labor and cost. Results based on a BioLamina distributed stem cell research protocol-survey designed for scientists, managers, directors and PI's (n=41) involved in the field of stem cell research, aimed to generate a better understanding of some of the issues involved in generating robust protocols for isolation, expansion and differentiation of human stem cells.

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2. Stem Cells: A Renewable Source of Mature Human Cell Types

The remarkable utility of stem cells for life science applications derives from their two defining properties: (1) their capacity for self-renewal, and (2) the ability to differentiate into multiple specialized cell types.

Pluripotent Stem Cells

Pluripotent stem cells (PSC) are highly versatile cells that have virtually unlimited capacity to proliferate in the undifferentiated state and are capable of differentiating into almost any somatic cell type. These properties make PSC very attractive as a renewable source of genetically identical cells for a wide variety of applications, enabling expansion of the cells to large batch sizes prior to differentiation.

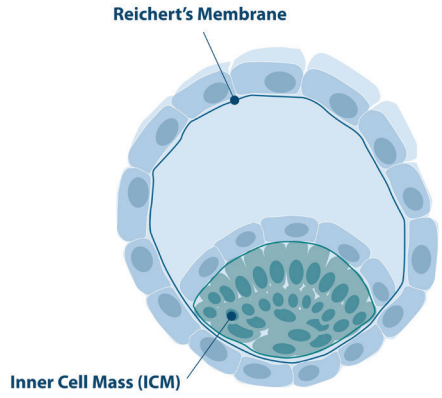

Figure 2: Laminins in the embryonic stem cell (ESC) niche. In the developing embryo, laminin 521 and 511 are among the first extracellular proteins to be expressed, already at the 2-4 cell stage embryo. Lamininare key cell adhesion proteins of the natural stem cell niche, expressed and secreted by the ESC in the inner cell mass (ICM) of the embryo, regulating cell fate.

There are two varieties of PSC available:

- **Embryonic stem cells (ESC)** arise naturally during embryo development and give rise to all cell types in the body. They comprise the inner cell mass of the blastocyst (Figure 2).
- **Induced pluripotent stem cells (iPSC)** are artificially created by reprogramming adult cells, such as skin fibroblasts, with transcription factors that maintain pluripotency by inhibiting differentiation. iPSC technology enables development of individualized disease models and treatments because they can be reverse engineered from adult patient samples. Hence, iPSC does not cause the ethical concerns as is associated with ESC.

Multipotent Stem Cells

Multipotent cells are so named because they have the potential to give rise to multiple, but not all, somatic cell types. In addition, they tend to have more limited capacity for self-renewal, which decreases the yield. Nevertheless, they are increasingly preferred for clinical applications in part because they carry less risk of teratoma formation compared to ESC and iPSC. Moreover, they are free of the ethical concerns associated with ESC, as well as any prior genetic manipulation (unlike iPSC).

- **Hematopoietic stem cells (HSC)** were among the first stem cell type used for human transplantations. They continue to be widely used in cancer therapy for bone marrow transplantation after chemotherapy. HSC are also becoming an important tool in immunotherapy, for autoimmune disorders and malignancies. In their native environment, HSC obtain vital cues from various extracellular matrix components including laminin, collagen and fibronectin.
- **Mesenchymal stem cells (MSC)** differentiate into cartilage, bone and fat cells, and are therefore especially useful in regenerative medicine. In the presence of serum or platelet lysate, MSC attach to plastic culture ware. However, when culturing in serum-free medium, for example when establishing a fully defined culture system, a matrix or substrate is needed to support attachment.
- **Cancer stem cells (CSC)** are another tool of interest for the development of novel targeted approaches for cancer therapy, although there are many practical challenges yet to overcome. Common CSC protocols do not specify use of a culture matrix, yet ***in vivo*** the CSC niche plays important roles in protecting CSC from the immune system, maintaining plasticity of phenotype, and facilitating metastatic potential.[1] Laminin in particular, is a key component of the CSC niche. For example, breast cancer stem cells produce a laminin 511 (LN511) matrix, which is critical for integrin-mediated signaling to maintain stemness.[2]

Quick Guide 1: Types of Stem Cells

STEM CELL TYPES (not comprehensive)			DERIVATION	DIFFERENTIATION POTENTIAL	NEEDS MATRIX/FEEDER SUPPORT	GENETIC MANIPULATION	ETHICAL CONCERNS	TERATOMA FORMATION	PATIENT-DERIVED
PLURIPOTENT	Embryonic Stem Cells	ESC	Embryonic tissue (inner cell mass of blastocyst)	Mesodermal, endodermal, ectodermal lineages	Yes	No	Yes	Yes	No
	Induced Pluripotent Stem Cells	iPSC	Adult (post-natal) tissues (e.g. fibroblasts); Genetically or chemically reprogrammed to express transcription factors that confer pluripotency	Mesodermal, endodermal, ectodermal lineages	Yes	Yes	No	Yes	Yes
MULTIPOTENT	Hematopoietic Stem Cells	HSC	Adult bone marrow, umbilical cord blood, peripheral blood	Myeloid and lymphoid blood cell lineages	Yes	No	No	No	Yes
	Mesenchymal Stem Cells	MSC	Fetal and adult tissues, e.g. bone marrow (BM-MSC), umbilical cord blood, adipose (AdMSC), molar, amniotic fluid	Mesodermal lineages: chondrocytes (cartilage), osteocytes (bone), adipocytes (fat); also some endodermal and ectodermal lineages	Yes/No (yes if cultured in serum-free medium. No If the medium contains serum)	No	No	No	Yes
	Cancer Stem Cells	CSC	Tumor tissue	Potentially all cell types in the tumor of origin	No	No	No	Yes	Yes

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3. The Extracellular Microenvironment

In their native environment, stem cells and most other cells in the body, do not exist in isolation. Instead, they form discrete tissues, and surround themselves with an extracellular matrix (ECM) self-assembled from a variety of secreted proteins and glycosaminoglycans (negatively charged polysaccharides). The extracellular microenvironment is highly dynamic and responsive, supporting complex signaling mechanisms via cell-matrix interactions, cell-cell interactions and soluble factors such as nutrients, growth factors, and cytokines.

Extracellular Matrix and Basement Membrane

A specialized form of ECM, called basement membrane (BM), forms a thin sheet that provides structural support, and also mediates vital signaling mechanisms critical for cell health, behavior and function. Structurally, BMs are composed primarily of covalently cross-linked networks of laminins and type IV collagens, bridged by adaptor proteins such as the heparan sulfate glycoproteins and nidogen (also known as entactin). BM protein expression and composition varies both developmentally and according to tissue type. Laminin isoform 521 is specific to BM of the stem cell niche ***in vivo***, and plays a crucial role in hPSC phenotype and function.

Quick Guide 2: Basement membrane proteins and their cellular receptors[3]

PROTEIN (family)	STRUCTURE	CELLULAR RECEPTORS
Agrin	Single-chain proteoglycan	Dystroglycan, MuSK / agrin receptor
Collagen IV	At least 3 heterotrimers formed from 6 homologous α chains	Integrins (α1bβ1, α2β1)
Collagen XVIII	Homotrimer proteoglycan, alternative splicing	Heparan sulfate proteoglycans (HSPGs)
Fibulin	5 isoforms; alternative splicing (fibulin-1); monomers and disulfide-linked dimer (fibulin-2)	Integrins (fibulin-2: αIIbβ3; fibulin5: aαVβ3, αVβ5, α9β1)
Laminin	At least 15 heterotrimers formed from 5 α chains, 3 β chains, and 3 y chains	Integrins (1β1, α2β1, α3β4, α6bβ1, α6β3, α7β1), dystroglycan, heparin sulfate proteoglycans; sul-fatides, HNK-1 (α1 chain), lutheran (α5 chain)
Nidogen/entactin	Single chain; 2 isoforms	Integrins (α3β1, αVβ3)
Perlecan	Single-chain proteoglycan	Dystroglycan

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In addition to forming BM between epithelial and stromal cell layers, ECM also surrounds cells to form a 3-dimensional scaffold called the interstitial matrix. The relatively broad term ECM thus refers to a complex and highly variable network of components including fibrous proteins (collagens and elastin), adhesive glycoproteins (such as laminin, fibronectin, nidogen, and tenascin), and glycosaminoglycans (including hyaluronic acid, chondroitin sulfate, dermatan sulfate, keratin sulfate, heparin and heparan sulfate). ECM is also rich in proteolytic enzymes, such as matrix metalloproteinases (MMPs), involved in breakdown and remodeling of the ECM.

Cell Adhesion Molecules Mediate Attachment of Cells to ECM

Cells attach to ECM networks by means of sulfated glycolipids and cell adhesion molecules (CAMs) on the cell surface. Interaction of CAMs with cognate ligands in the ECM and on neighbouring cells activates intracellular signalling pathways important for a variety of processes, including gene expression, survival, migration, proliferation and differentiation. The most important CAM families for hPSC maintenance and self-renewal are thought to be integrins and cadherins.

Integrins

The integrin family of transmembrane receptors plays a major role in transducing signals both to and from the extracellular microenvironment, enabling cells to quickly adapt and respond to extracellular events. Integrins are heterodimers made up of two glycoprotein subunits, α and β. As many as 18 α chains and 8 β chains have been found, combining to form 24 different types of integrin. The ligand specificity and affinity of each type varies depending on its constituent α and β chains. Integrin ligands present in ECM include collagen, fibronectin, laminin, vitronectin, E-cadherin and MMP.

In hPSC culture, integrin types and expression levels vary, depending in part upon which culture substrate you select. Data from various studies suggest that laminin-specific α6β1 integrin is expressed at relatively high levels in hPSC, and plays a key role in survival and self-renewal[7]. A variety of other integrins including αVβ5, αVβ3 and α2β1 are also thought to be important for hPSC attachment to vitronectin, fibronectin and collagen.[4]

Quick Guide 3: Integrin extracellular ligands[5]

LIGAND	INTEGRIN
Adenovirus penton base protein	αvβ3, αvβ5
Bone sialoprotein	αvβ3, αvβ5
Borrelia burgdorferi	αIIbβ3
Candida albicans	αMβ2
Collagens	α1α1, α2α1, α11β1, αIbβ3
Denatured collagen	α5β1, αvβ3, αIIbβ3
Cytotactin/tenascin-C	α8β1, α9β1, αvβ3, αvβ6
Decorsin	αIIbβ3
Disintegrins	αvβ3, αIIbβ3
E cadherin	αEβ7
Echovirus 1	α2β1
Epiligrin	α3β1
Factor X	αMβ2
Fibronectin	α2β1, α3β1, α4β1, α4β7, α5β1, α8β1, αvβ1, αvβ3, αvβ5, αvβ6, αvβ8, αIIbβ3
Fibrinogen	α5β1, αMβ2, αvβ3, αxβ2, αIIbβ3
HIV Tat protein	αvβ3, αvβ5
iC3b	αMβ2, αxβ2
ICAM-1	αLβ2, αMβ2
ICAM-2,3,4,5	αLβ2
Invasin	α3β1, α4β1, α5β1, α6β1
Laminin	α1β1, α2β1, α3β1, α6β1, α6β4, α7β1,
MAdCAM-1	α4β7
Matrix metalloproteinase-2	αvβ3
Neutrophil inhibitory factor	αMβ2
Osteopontin	αvβ3
Plasminogen	αIIbβ3
Prothrombin	αvβ3, αIIbβ3
Sperm fertilin	α6β1
Thrombospondin	α3β1, αvβ3, αIIbβ3
VCAM-17	α4β1, α4β7
Vitronectin	αvβ1, αvβ3, αvβ5, αIIbβ3
von Willebrand factor	αvβ3, αIIbβ3

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Cadherins

Cadherins are a large family of calcium-dependent adhesion molecules that mediate both cell-cell and cell-matrix interactions. The main type expressed by hPSCs is E-cadherin, which facilitates intercellular adhesion and colony formation.[6] A chemically defined matrix comprising a mix of laminin 521 and E-Cadherin has been demonstrated to support clonal derivation, clonal survival and long-term self-renewal of hESC under xeno-free conditions without the need for ROCK inhibitors.[7]

The stem cell niche

In the body, stem cells reside in highly specialized and sensitive microenvironments, or "niches", which serve to protect and maintain the cells in a quiescent state, primed to respond to the needs of surrounding tissue (Figure 3). Naturally, when culturing stem cells ***in vitro***, it makes sense to replicate their native stem cell niche as closely as possible to maintain their authentic phenotypes and functions. The culture matrix is therefore a crucial element in establishing any stem cell culture system.

Embryonic stem cell lines are derived from the inner cell mass of blastocyst stage embryos (Figure 1). Within this specialized niche, pluripotent stem cells naturally express and secrete laminin 521 and/or 511 isoforms, which are key components of the BM that supports their pluripotency and self-renewal. [7, 11] At the same time, they co-express cognate integrins (specific for laminin α5 isoforms) at the cell surface to mediate BM attachment and important signaling cascades. In culture, both hESC and hiPSC express these laminin isoforms [9, 10] and matching integrin receptors, so they are naturally equipped to interact with and thrive on culture surfaces coated with laminin 521/511. Laminin 521 and 511 therefore provide biologically matrices that closely mimic the natural stem cell niche (see section 4 for more detail on laminin nomenclature and isoforms).

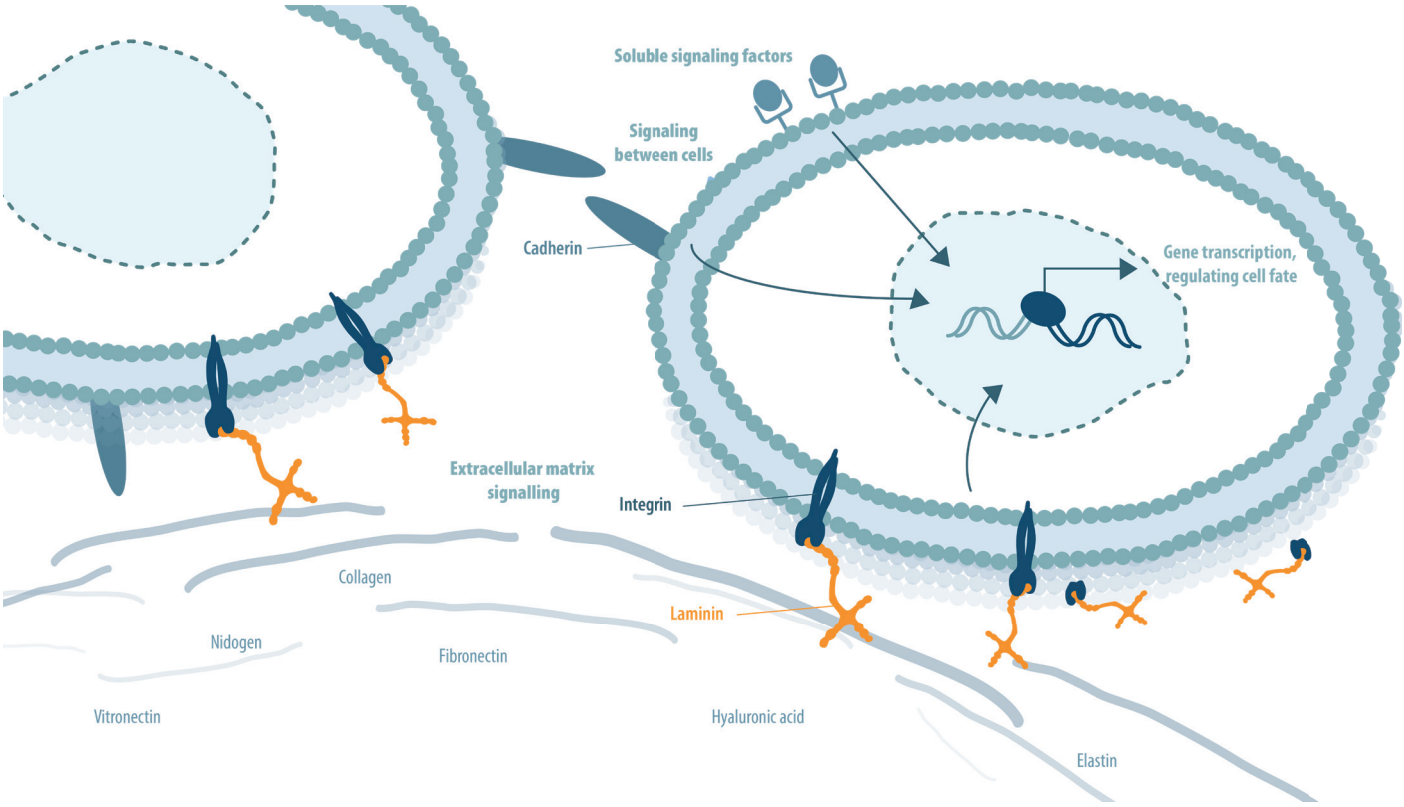

Figure 3. Stem cells interactions with the extracellular microenvironments and its effect on cell behavior. Stem cell fate is influenced by coordinated interaction of soluble factors, extracellular matrix (ECM) and signals from neighboring cells. The multi-faceted cell-ECM communication takes place through both integrin and non-integrin membrane-bound receptors and induces complex intracellular signaling pathways with subsequent effect on survival,self-renewal, migration, morphogenesis and differentiation.

4. Stem Cell Culture Systems

Most stem cell types, including PSC, will not adhere directly to standard plastic and glass culture ware. At a minimum, they require some sort of substrate or matrix for integrin-mediated anchorage. However, as detailed in section 3, culture matrices do a lot more than provide substrates for attachment. They play essential roles in regulating key processes such as cell survival, proliferation, differentiation and migration. The wrong type of matrix can increase the chances of spontaneous differentiation and thereby irreversibly alter the cells' properties.

As stem cell technologies have evolved over the years, the available matrices and individual component substrates have become more defined, more reproducible and a better representation of the various stem cell niches found *in vivo*.

Feeder-Dependent Cultures

Early stem cell culture systems relied on co-culturing with "feeder" cells to condition the medium, establish an extracellular matrix and help prevent the stem cells from losing their pluripotency. The most commonly used feeder cells for PSC culture are mouse embryonic fibroblasts (MEF). Various human feeder cell types are also available. Feeder cells are typically prepared as a monolayer, which is then mitotically inactivated, either by irradiation or the addition of an inhibitor, to prevent overgrowth in long-term culture. Stem cells are then seeded on top of the feeder layer.

A number of limitations and drawbacks make feeder-dependent systems less suited for stem cell culture:

- Labor-intensive and time-consuming to establish
- Poorly defined, unpredictable and challenging to reproduce consistently
- Primary MEFs are typically heterogeneous and lose ability to support PSC proliferation over time
- Growth characteristics difficult to control
- Risk of secretion of factors like TGFβ, BMP-4 and Activin A in the culture medium
- Overgrowth of the feeder layer can lead to nutrient depletion, which limits expansion of the stem cell population
- Most require supplementation with βFGF to maintain PSC pluripotency, which adds an extra element of complexity into the system
- Potential source of pathogens and allergens
- Often animal-derived, which is prohibitive for various pharmaceutical and clinical research applications where safety and compliance guidelines specify xeno-free

Feeder-Free Cultures

More recently, natural and synthetic matrices have been utilized to replace feeder co-cultures. Matrices used to support feeder-free stem cell culture can be classed as undefined (typically derived from crude ECM extracts), semi-defined (using proteins and other components purified from native sources), or fully defined (comprising recombinant and/or synthetic components). For a fully defined culture system, defined matrices must be used in combination with defined serum substitutes and supplements, such as recombinant growth factors and cytokines.

In general, feeder-free cultures are more amenable to scale-up than feeder-dependent systems. Successful feeder-free systems must strike a balance between authenticity and reproducibility. Simply put, they must be complex enough to be biologically representative of the natural stem cell microenvironment, while having a minimal number of well-defined components so that they are robust and reproducible. When producing human PSC for various clinically related applications, it is preferable to use only human (xeno-free) components in the culture system, in order to ensure safety and the highest degree of biological relevance.

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Undefined Matrices: ECM Extracts

Some of the first feeder-free stem cell cultures were established using crude ECM extracts. Although these extracts are often described as "natural", the most common source is abnormal tumor tissue, such as Engelbreth-Holm-Swarm murine sarcoma (mEHS). Commercial EHS extracts, such as Matrigel and Geltrex, are gelatinous mixtures rich in mouse ECM basement membrane proteins. They often contain a mixture of ECM components, such as laminin 111, which may not be representative of the native stem cell niche. Although they support expansion and differentiation of PSC, EHS extracts also contain variable levels of tumor growth factors, which can have deleterious and unpredictable effects on PSC cultures, including triggering spontaneous differentiation. Xeno-free human ECM extracts, for example derived from human placenta or established hESC lines, are now available, although generally more expensive and therefore less widely used.

Whether animal-derived or xenobiotic-free, ECM extracts tend to be poorly characterized, and can vary significantly in composition and performance from lot to lot. For this reason, some manufacturers offer a lot reservation service for large volumes. Nevertheless, this inconsistency can cause significant problems when trying to establish robust production processes. Protocols for working with ECM extracts are often time-consuming and tricky to execute properly. Thawing, dilution and coating procedures are a common source of contamination and variability in PSC cultures. Commercial ECM extracts are increasingly being made available in a range of quality grades, including growth factor reduced and ready-to-use forms qualified for non-clinical hESC applications.

Semi-defined Matrices: Purified Components

Better-defined PSC culture systems can be achieved using adhesion proteins and other components purified from native sources, such as plasma or placenta. While purified components are a step forward in creating defined matrices, it can be difficult or impossible to purify large multifunctional proteins like laminin with all of their functions intact.[8] Moreover, even after purification, these components may contain pathogens, cytokines, growth factors or other contaminants at undefined levels, potentially in amounts sufficient to induce phenotypic and behavioral alterations in the stem cell population.

Purified substrates may be used singly or in combination with other components to create better-defined, application-specific culture systems. Purified components must be carefully selected and optimized for the intended application. Not all matrix components are equal in terms of their biological relevance or ability to support expansion and maintenance of PSCs in the undifferentiated state. Likewise, various components differ in their capacity to support differentiation down desired lineage pathways and subsequent maintenance of specialized cell types.

Common proteins purified from native sources for use in PSC culture are summarized in Quick Guide 3, and include:

Laminin, purified

Laminin is the major non-collagenous protein component of BM. The laminin family of heterotrimeric glycoproteins includes at least 16 different isoforms, named according to their component α, β and γ chains. For example, laminin isoform 521 is made up of one α5, one β2 and one γ1 chain. Within BM, laminins form web-like networks that support cell adhesion through integrin-mediated associations. LN521 promotes cell adhesion of most primary cell types and efficiently support PSC adherence and maintenance of pluripotency, proliferation, migration and differentiation.[10, 12] In contrast, LN111 is primarily expressed in extra-embryonic BM, where it stimulates cells to commit to differentiation. Purified laminins are available from a number of vendors, and typically contain an indeterminate mixture of laminin isoforms. Sigma-Aldrich, for example, offers laminins purified from various sources including mEHS sarcoma BM, human placenta and human cell line co-cultures. The presence of LN111 and other isoforms non-representative of the natural stem cell niche could contribute to spontaneous differentiation and other unpredictable behaviours in culture.

Collagen

Collagen is the most abundant glycoprotein component of ECM. There are 30 different types of collagen, with varying tissue distributions. Collagen type IV predominates in BM, and has been implicated in directing mouse and human ESC down mesodermal lineages.[13, 14, 15, 16] Fibrillar collagens (Types I, II, III, VI, XI) are variously expressed in interstitial ECM throughout the body, with type I being the most abundant.

Fibronectin

Fibronectin is a major glycoprotein component of ECM deposited ubiquitously throughout the body. Hepatocytes also secrete a soluble form of fibronectin directly into plasma. Widely used in cell culture to promote adhesion of many cell types, fibronectin is deposited ***in vivo*** as a dimer composed of disulfide-linked 230-270 kDa subunits that are assembled into fibrillar networks, which serve important structural and functional roles during development and in the adult body. Each fibronectin subunit is actually a mosaic of three types of repeating sequence (I, II and III), which mediate binding to other fibronectin molecules, the ECM, and cell surface receptors. Fibronectin has been found to provide adequate support for PSC adherence and maintenance of pluripotency. However, because PSC interaction with fibronectin depends on multiple domains, the integrity of purified fibronectin is an important consideration. For example, a comparative study of various culture matrices found that expression levels of genes important for pluripotency varied in hPSC grown on fibronectin, depending on the source of the fibronectin.[17]

Vitronectin

Vitronectin is another adhesive glycoprotein found abundantly in ECM deposited by many cell types. Secreted as a 75 kDa monomer or clipped disulfide-linked dimer, vitronectin promotes cell adhesion, proliferation, migration and differentiation of some cell types. It is also abundant in platelets, and plays a role in hemostasis. At sufficient concentrations (at least 250ng/cm^2), vitronectin coatings support maintenance of hPSC phenotype and pluripotency.[18] Vitronectin has multiple integrin and heparan sulfate proteoglycan (HSPG) binding sites, which must be preserved during purification for full functionality.

Fully Defined Matrices: Recombinant and synthetic components

For reliable production of stem cells and stem cell-derived products, fully defined culture systems are essential. Clinical therapeutic and pharmaceutical applications in particular, often require an additional level of rigor to ensure safety and biological relevance for humans. Recombinant matrix proteins and other synthetic substrates have the advantage of being fully defined and pathogen-free, with low batch-to-batch variability. A wide selection of animal component-free human recombinant ECM proteins and protein fragments are now commercially available.

It is important to note that quality and compliance of recombinant products may vary, ranging from research-use-only to cell therapy-grade. Another important consideration is whether to use a full-length protein or a truncated version. While recombinant fragments may support adhesion, pluripotency, and self-renewal, they may lack functional domains whose importance functional performance may not yet be fully understood. In addition, for optimal performance they may require specialized culture media with proprietary formulations, which can make further optimization of the culture system difficult.

Examples of recombinant products widely used to support hPSC culture and differentiation are summarized in Quick Guide 4. Three of the most commonly selected are:

Laminin, recombinant

In recent years, recombinant laminins have become a preferred substrate for fully-defined matrices, particularly for clinical stem cell applications.[19] A variety of human recombinant laminins are now commercially available to support cell culture, including hPSC expansion, lineage specification and maintenance of differentiated cell types. BioLamina's expansive portfolio of 9 different

laminin isoforms currently provides the only original, full-length, human, recombinant laminins on the market. Among them, recombinant human laminin 521, Biolaminin™ 521, is the most biologically relevant for mimicking the authentic embryonic stem cell niche in culture and is ideally suited for use with both hESC and hiPSC cultures. Via strong interaction to the α6β1 integrin, LN521 affect the PI3K/Akt and the focal adhesion kinase (FAK) signalling pathways, linked to hPSC self-renewal and expression of stemness. Due to the biologically relevant support from the Biolaminin 521 LN (LN521) substrate, it can be used on its own as a single-component matrix to provide full support for PSC expansion and differentiation. LN521 encourages PSC to grow in a homogeneous monolayer, without the need to manually remove differentiated cell areas. The cells are easily passaged with or without enzymatic reagents, and without the need for apoptosis inhibitors, such as ROCKi (Figure 4). Section 5 provides more detail on the Biolamina laminin portfolio.

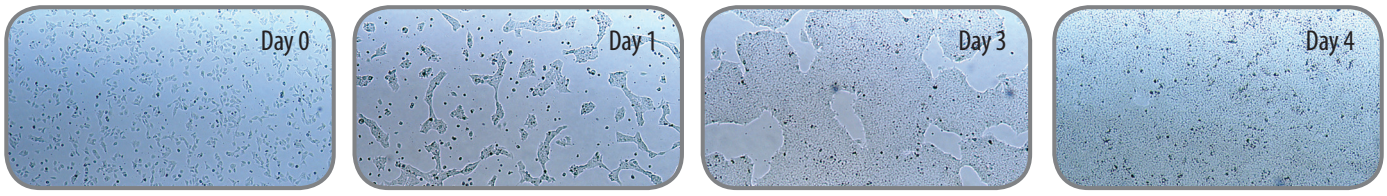

Figure 4: Human ES cell culture morphology on LN521. Day 0: hESC HS181 seeded as single cells on LN521, no apoptosis inhibitors (ROCKi) needed. 1 hour after seeding, the cells have attached and are evenly distributed over the plate. Day 1: the cells have formed small colonies. Day 3: The cells rapidly proliferate, without sharp boarders between different colonies. Day 4: The cells have formed a homogenous cell monolayer. The cells can be cultured to near confluence without signs of spontaneous differentiation.

Fibronectin, recombinant

Recombinant fibronectin remains another popular choice for fully defined culture systems, not least because the use of recombinant fibronectin aids transition to defined conditions from protocols already established using purified fibronectin, which has compared favorably to laminin in some studies.[17, 20, 21] Various recombinant fibronectin fragments comprising integrin binding domains have been investigated as candidates for synthetic substrates, but results have suggested that that multiple domains are required to support hPSC expansion and differentiation in culture.[22] Both full length and recombinant fibronectin forms are available commercially.

Vitronectin, recombinant

Both full-length and truncated forms of recombinant human vitronectin are commercially available. A 2008 investigation comparing performance of various ECM substrates highlighted recombinant human vitronectin as a viable functional alternative to Matrigel in supporting self-renewal and pluripotency of hESC cell lines.[23] Notably, the study did not include analysis of α5-laminins, which are now recognized as signature components of the natural stem cell niche, and have been proven to be critical for PSC survival and self-renewal.[24] More recently, recombinant truncated vitronectin (amino acids 62-478) was found to be superior to wild type vitronectin in supporting hPSC attachment and survival, when used on conjunction with specialized culture medium.[25]

5. Laminins: Biologically relevant support for cell culture

Laminin (LN) is a key ECM protein that acts as an adhesive and maintains the structure of the body. More importantly, laminin mediates vital signaling between cells and their microenvironment. These cues are crucial during development, and for maintenance of cell health and function in the adult. Different cells and tissues each have their own specific laminin composition. When recreating the extracellular microenvironment for ***in vitro*** cell culture, it can be critical to choose the right laminin to support cell health, proliferation, differentiation and function.

BioLamina produces and distributes a wide selection of chemically defined and animal origin-free laminin isoforms to facilitate diverse applications, including reliable expansion of pluripotent cells, as well as differentiation and maintenance of specialized cell types, such as hepatocytes, skeletal muscle cells and different neural cells. The impact of BioLamina's laminin matrices on cell culture quality has been scientifically validated in many high-impact journals.

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CULTURE SYSTEM			COMPONENTS	TYPICAL SOURCES	EXAMPLE COMMERCIAL PRODUCTS	PROS	CONS
FEEDER-DEPENDENT	UNDEFINED	Feeder cells	Typically mitotically inactivated fibroblasts cultured on gelatin-coated plates.	Mouse Human	MEF, HFF, SNL, STO	• Long-established methodology • Validated protocols	• Undefined matrix components and growth factors • Xenogenic/animal origin, source of allergens and pathogens, • Cellular cross-contamination, overgrowth of the feeder layer, • High variability, • Poor reproducibility, • Heterogeneous cultures, • Labor-intensive protocols
FEEDER-FREE	UNDEFINED	Basement membrane extracts ("natural")	Basement membrane extract (BME) from animal-derived tumor tissue such as murine Engelbreth-Holm-Swarm (mEHS) sarcoma. Some human sources also available, e.g. from human placenta.	mEHS (tumor tissue) Placenta	Matrigel (Corning), Geltrex (ThermoFisher Scientific), Cultrex (Trevigen),	• Easier to use and more reproducible than feeder-dependent cultures. • Easier to scale for mass production. • Available in several grades/qualities including "hESC-qualified", ready-to-use, reduced growth-factor etc.	• Commonly derived from biologically irrelevant tumor tissue, • Poorly defined, animal origin complicates use for human applications, • Batch-to-batch and lot-to-lot variability, concentration of growth factors and soluble components may vary, • Thawing and reconstitution procedures are time-consuming and subject to operator-dependent variability, • Risk of contamination (most cannot be sterile filtered before use; not suitable for clinical setting). • High laminin 111 content inappropriate for reliable PSC survival and expansion
	SEMI-DEFINED	**PURIFIED FROM NATIVE SOURCES**					
			Laminin	mEHS tumor, cultured human cell lines (e.g. fibroblast and epithelial cell co-cultures), placenta	Laminin from EHS murine sarcoma BM (Sigma), Ultrapure Laminin (Corning)	• Better defined compared to BME • Human sources available	• Some components and isoforms less biologically appropriate than others. • May contain impurities or fragments, • Animal origin complicates use for human applications, • Fractionated laminin molecules isolated from tissue lack domains required for function formation of proper extracellular network
			Fibronectin	Plasma (bovine, human, etc.)	Fibronectin bovine plasma (Sigma-Aldrich), Human Plasma Fibronectin Purified Protein (Sigma-Aldrich), Human Plasma Fibronectin (Corning), Human Fibronectin (ThermoFisher Scientific)		
			Vitronectin	Plasma	CellAdhere Vitronectin (Stem Cell Technologies)		
			Collagen IV	Human placenta	Human Collagen IV (Corning)		
			Collagen I		CellAdhere Collagen I (Stem Cell Technologies), Human Collagen I (Corning)		
	FULLY DEFINED	**RECOMBINANT** (purified from various hosts, e.g. HEK293, E.Coli) **OR SYNTHETIC**					
			Laminin isoforms - full length	Recombinant	Recombinant Human CT521, MX521, LN521, LN511, LN421, LN411, LN332, LN221, LN211, LN121, LN111 (BioLamina). ThermoFisher Scientific, Corning and Stem Cell Technologies, have included BioLamina's LN521 as a high-end stem cell matrix in their product portfolios.	• Fully defined components provide better control and reduce sources of variability • Pathogen-free.	• Quality/grade may vary depending on recombinant technology and supplier (e.g. endotoxin testing required), • Recombinant fragments and synthetic constructs may lack important functional and structural domains, • May not mimic natural environment as intended (more uncertainty), • May be modified with non-native sequences, such as His tag for purification, • May require use of specialized proprietary media, • Can be expensive
			Laminin isoforms - fragment	Recombinant	iMatrix-511 (Matrixome, Reprocell)		
			Vitronectin - full length	Recombinant	Vitronectin-XF (Stem Cell Technologies, Primogen)		
			Vitronectin - fragment	Recombinant	VTN-N (ThermoFisher Scientific), CTS-Vitronectin (ThermoFisher Scientific)		
			Fibronectin - full length	Recombinant	Fibronectin human (Sigma-Aldrich)		
			Fibronectin - fragment	Recombinant	Fibronectin Fragment III-C human (Sigma-Aldrich), Fibronectin (FN1) human (MyBioSource), Recombinant His-tag Fibronectin Protein (Sino Biological)		
			E-Cadherin - fragment or IgG Fc fusion	Recombinant	Recombinant Human E-Cadherin Protein (R&D Systems), StemAdhere Defined Matrix (Stem Cell Technologies), His Tag CDH1 rh Protein (Thermo Fisher Scientific), E-Cadherin human (Sigma-Aldrich)		
			Snythetic surfaces with peptide conjugates	Synthetic/recombinant	"PureCoat ECM mimetics (Corning), Synthemax (Corning)"		• Formulations with unpublished components/composition are an unknown 'black box' that adds an element of risk, • Biological relevance for the chosen cell type may be unknown, • Lack of information confounds trouble-shooting and optimization, • Can be expensive
			Proprietary single recombinant human or humanized protein	Proprietary recombinant	CTS CellStart (ThermoFisher)		
			Proprietary mixture of recombinant human adhesion molecules	Proprietary recombinant	StemXVivo (R&D Systems), Mesencult-SF (Stem Cell Techologies)		

Biolaminin 521: biological relevance and superior for hPSC culture performance

Biolamina's original Biolaminin™ 521 research grade product, LN521, is the company's flagship product, the culmination of years of research to develop the most biologically relevant matrix for stem cell culture and differentiation. Available in several grades to support the development process from discovery through to manufacture for clinically related applications, recombinant human LN521 biologically relevant and is scientifically proven to be effective for maintenance of stem cells (hESC and hiPSC), as well as many other cell applications (Figure 5).

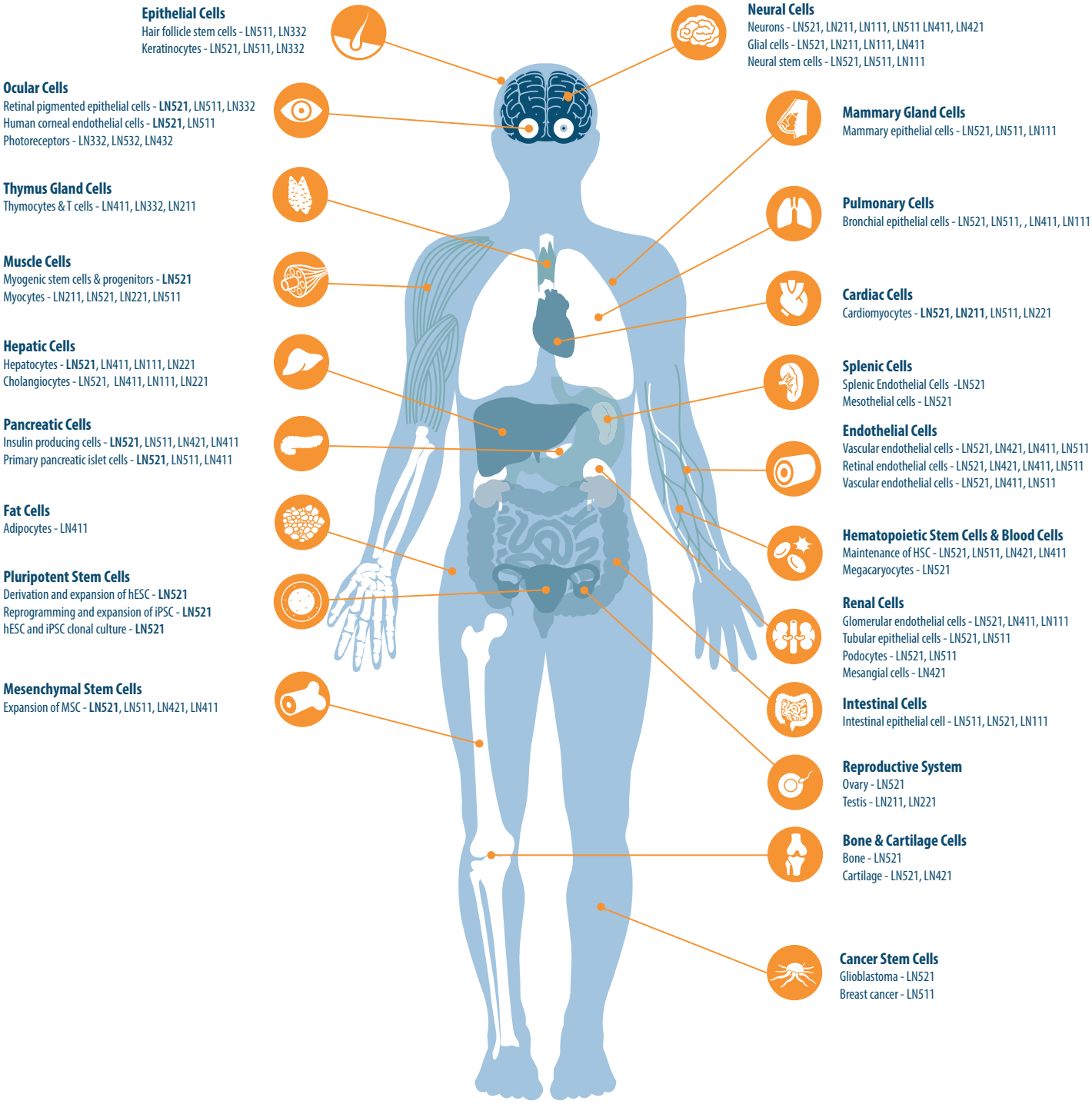

Figure 5: Laminin isoform expressed in different human cells and tissues. Laminins are key components of the basement membrane which adhere to the cells, maintain the subcultural organization of the basement membrane and play an essential role in the regulation of cell behaviour.

Key advantages of using LN521 for hPSC culture and differentiation include:

- Biologically relevant culture environment - mimics the authentic stem cell niche
- Chemically defined and animal origin-free culture matrices
- More consistent and reliable performance, with no lot-to-lot variability
- Homogeneous and genetically stable hPSC cultures
- Easy single-cell or colony passage without need for ROCK inhibitors
- Faster expansion with no spontaneous hPSC differentiation
- More efficient differentiation - enhanced cell maturity, polarization and organization
- Supports weekend-free feeding
- Simplified and reliable protocols
- Full traceability documentation

From concept to clinic: key considerations for translational stem cell applications

Progressing a cell therapy application from concept to manufacture for use in a clinical setting can present varied challenges, as the application is prototyped, optimized, scaled up, and qualified for manufacture and clinical use. In the early phases of discovery and prototyping, a wide range of culture conditions and other variables may need to be tested at reasonably high throughput. Culture systems need to be flexible, reproducible, easy to use, and able to support a high expansion rate, while at the same time ensuring homogeneous monolayer growth, maintenance of pluripotency, and genetic stability - a tall order to say the least!

The culture matrix you choose can have a big effect in terms of the amount of time and manual effort required to feed and passage your stem cell cultures. For example, many matrices do not support weekend-free feeding regimes, so staff will need to be available at weekends to keep the cells going. In addition, cells can adhere very tightly to some matrices, which can make passaging difficult, decrease yields, and necessitate the use of enzymes and apoptotic inhibitors like ROCKi. It is also important to consider how amenable the culture system is to automation, which may be required for downstream scale-up, but may also bring benefit even at the early stages of development—to minimize experimental variability, human error and chances of contamination. Moving into pre-clinical and clinical stages, scale-up and automation are likely to become essential, as will the ability to demonstrate that all culture system components are animal origin-free. To qualify as cell therapy grade, components must be designed for clinical research, satisfying USP Chapter 1043 guidelines. Process control and traceability documentation is critical for handover to manufacturing.

To accommodate changing needs as you transition your culture system through each phase, BioLamina has developed three grades of its Biolaminin 521 product (Figure 6). All of them are full-length, human, recombinant laminin 521 substrates - the only ones on the market. Biolaminin 521 CTG is the world's first cell therapy grade laminin product and can support scientists throughout their cell therapy development process.

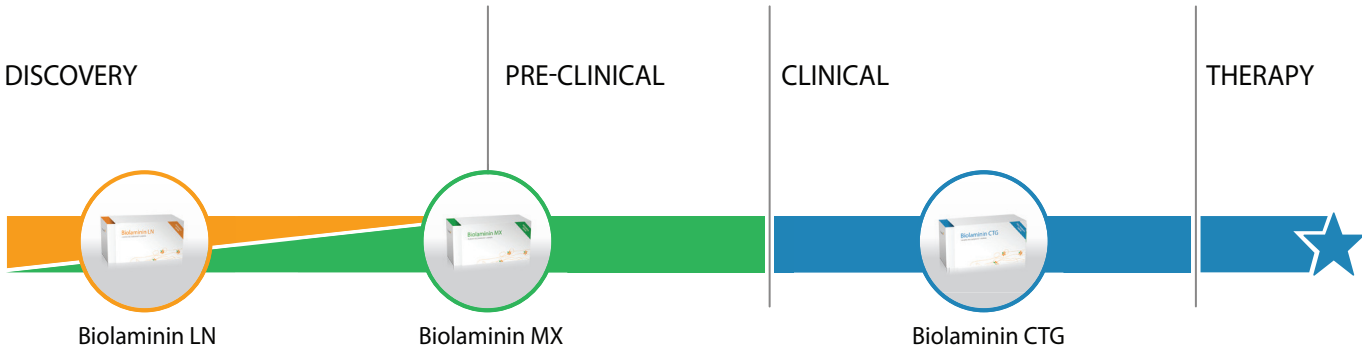

Figure 6: Biolaminin product range from discovery to clinic. Scientists who are at an early research stage will enjoy a seamless journey from basic research to post-approval therapy and production – starting with Biolaminin 521 research grade products (LN or MX), upgrading to the cell therapy grade product, Biolaminin 521 CTG, for the clinical development phases.

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This new product complements BioLamina's renowned original research grade laminin product, Biolaminin 521 LN (LN521). As a complement to the CTG product, Biolaminin 521 MX has been developed, facilitating a seamless transition from discovery into clinical settings.

Quick Guide 5: Table comparing specifications of the Biolaminin 521 LN, MX and CTG cell culture productss

	Biolaminin 521 LN	Biolaminin 521 MX	Biolaminin 521 CTG
Research grade substrate	✓	✓	
Cell therapy grade substrate			✓
Designed for clinical research			✓
Manufacturing control and traceability			✓
Animal orign-free	✓	✓	✓
Animal-origin free to 2o Level		✓	✓
Easily adaptable to automation	✓	✓	✓
Defined	✓	✓	✓
Scientifically proven	✓	✓	✓
Biologically relevant for hPSC	✓	✓	✓
Homogeneous and genetically stable hPSC	✓	✓	✓
Easy and flexible culture system	✓	✓	✓
Low variability	✓	✓	✓
Efficient differentiation and enhanced cell maturation, polarization and organization	✓	✓	✓
Positive effect on stabilizing and homogenizing pluripotent gene expression profiles between hESC lines	✓	N/A	N/A

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