BioLamina application areas: Key publications

To send as links to customers or collaborators. By application or method.

Every application area comes with an **AppNote** or **Instruction**.
See pdf document “BioLamina material_Links_AppNotes and Instructions”

Contents

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1) Methods

1.1.) Biolaminin® for gene editing

1) Biolaminins® for gene editing incl Crispr-Cas9 application

LN521 coating supported high throughput hPSC clonal selection and expansion. Single cell cloning efficiency were increased compared to commonly used ECMs such as Matrigel or Geltrex. In addition, the purity of Laminin 521 was found an advantage for imaging purposes (Vallone et al. 2020). For CRISPR/Cas9 protocols on hESC, LN521 coating was supporting the single cells seeding at a very low density (Kim et al. 2017 and Carlson-Stevermer et al. 2015). Biolaminin 521 can support cell survival at a seeding density as low as 5,000 cells/cm^2.

Vallone et al. 2020: Methods for Automated Single Cell Isolation and Sub-Cloning of Human Pluripotent Stem Cells - Vallone - 2020 - Current Protocols in Stem Cell Biology - Wiley Online Library

Kim et al. 2017: Generation of a Nrf2 homozygous knockout human embryonic stem cell line using CRISPR/Cas9 - ScienceDirect

Carlson-Stevermer et al. 2015): High-Content Analysis of CRISPR-Cas9 Gene-Edited Human Embryonic Stem Cells - PMC (nih.gov)

1.2.) User format modalities (Scale, Formats)

1.2.1.) Biolaminin for microcarrier coating

Lam et al. 2015 coated microcarriers with LN521 for hESC line (HES-3) culture and achieved a cell attachment (87%) and spreading (85%), which lead to high cell yields (2.4–3.5 x 10^6 cells/mL) within 7 days.

Eicke et al. 2018 seeded iPSC on LN521-caoted microcarriers and large numbers of megakaryocytes were produced after a 22 day differentiation protocol.

Sivalingam et al. 2016 reported the development of a serum-free, xeno-free, and chemically defined microcarrier-based platform using human LN521 for hPSC expansion, EB formation, and subsequently hematopoietic differentiation of hPSC. The microcarrier-based method resulted in significantly improved mesoderm induction, in a 6-fold improvement in hematopoietic precursor expansion and in a 80-fold improvement in the yield of red blood cell generation, compared to a conventional EB-based differentiation method. In a follow-up study Sivalingam et al. 2021 developed a scalable suspension agitation culture platform for differentiating hiPSC-microcarrier aggregates into functional red blood cells.

Yu et al. 2022 demonstrated as well that LN521 coated microcarriers serve as a scalable platform in a scalable perfusion bioreactor system to produce functional red blood cells from hiPSCs.

Lam et al. 2015: Improved Human Pluripotent Stem Cell Attachment and Spreading on Xeno-Free Laminin-521-Coated Microcarriers Results in Efficient Growth in Agitated Cultures | BioResearch Open Access (liebertpub.com)

Sivalingam et al. 2016: Superior Red Blood Cell Generation from Human Pluripotent Stem Cells Through a Novel Microcarrier-Based Embryoid Body Platform | Tissue Engineering Part C: Methods (liebertpub.com)

Sivalingam et al. 2021: A Scalable Suspension Platform for Generating High-Density Cultures of Universal Red Blood Cells from Human Induced Pluripotent Stem Cells - PMC (nih.gov)
Yu et al. 2022: Selection of O-negative induced pluripotent stem cell clones for high-density red blood cell production in a scalable perfusion bioreactor system - PMC (nih.gov)

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2)Applications – cell type specific

2.1.) Eye

2.1.1.) Biolaminin for corneal cells

The basement membrane of the corneal endothelium, also called Descemet's membrane, is composed of type IV collagen, type VIII collagen, laminin 332, laminin 411, and laminin 511, laminin 521 perlecan and nidogens (Massoudi et al., 2016, Kabosova et al., 2007; Byström et al., 2007; Okumura et al., 2015).
Kabosova et al., 2007: https://iovs.arvojournals.org/article.aspx?articleid=2183690
Byström et al., 2007: https://link.springer.com/article/10.1007/s00418-007-0288-4
Okumura et al., 2015: https://doi.org/10.1167/iovs.14-15163
Massoudi et al., 2016: https://link.springer.com/article/10.1007/s00441-015-2233-5

Both LN511 and LN521 have been shown to provide an excellent substrate for the culture human corneal endothelial cells.
Okumura et al. developed a protocol for the efficient in vitro expansion of human corneal endothelial cells on LN511 and LN521.
Okumura et al. 2015: Laminin-511 and -521 Enable Efficient In Vitro Expansion of Human Corneal Endothelial Cells | IOVS | ARVO Journals

Zhao et al showed that *in vivo* coating with LN511 enhances the adhesion and promotes the functional regeneration of the grafted corneal endothelial cells (CECs), improving the therapeutic function for corneal endothelial dysfunction.
Zhao et al., 2020: Laminin 511 Precoating Promotes the Functional Recovery of Transplanted Corneal Endothelial Cells | Tissue Engineering Part A (liebertpub.com)

Additionally, in a recent study, primary human corneal endothelial cells (HCEnCs) from old donors were sustained on LN511 and maintained a polygonal morphology, expression of critical markers and cell functions for several passages.
Merra et al. 2024: Impact of culture media on primary human corneal endothelial cells derived from old donors - ScienceDirect

Several eye conditions, such as keratoconus, bullous keratopathy, and Fuch's corneal dystrophy are, are related to the disruption of Bowman's membrane. In most keratoconus corneas, the Descemet's membrane (DM) contains laminin 332, normally only found in the epithelial basement membrane. in addition to laminin 511 normal corneas (Byström et al. 2007).

2.1.2.) Biolaminin for retinal cells

The beta-2 chain of the laminin molecule is important for the RPE cells (Libby, 1996). It surrounds RPE cells at the onset of rod photoreceptor birth and is present on the apical surface of the retinal neuroepithelium. Multi-layered extracellular matrix underlying the retina, Bruch's membrane, contains several laminins that retinal pigment epithelial cells grow on. The main isoforms expressed are laminin 521, 511, 332 and 111. Aisenbrey et al 2006 has shown that RPE cells adhere robustly to all laminins of Bruch´s membrane and actively synthesize these laminins.
Aisenbrey et al., 2006: https://iovs.arvojournals.org/article.aspx?articleid=2163683

Plaza Reyes et al. 2020 established an efficient, robust, direct and scalable xeno-free and defined monolayer differentiation protocol, where culture on supportive human recombinant LN111 and LN521 eliminated the need for manual selection, allowing large-scale production of pure hPSC-RPE.
Plaza Reyes et al., 2020: https://www.nature.com/articles/s41467-020-15326-5?error=cookies_not_supported&code=fd32c8c7-f0c9-4038-a3ad-ee55928bbe3c

Hongisto et al. 2017 described a robust animal origin- and feeder cell-free culture system for undifferentiated hPSCs along with efficient and scalable methods to derive high-quality retinal pigment epithelial (RPE) cells and corneal limbal epithelial stem cells (LESCs) on LN521.
Hongisto et al., 2017: https://stemcellres.biomedcentral.com/articles/10.1186/s13287-017-0738-4

LN521 also supports the culture of human primary RPE cells. Chen et al. show that culturing of human fetal RPE cells on laminin 521 PET transwell inserts creates a model that recapitulates the structural, molecular and apical/basolateral signatures of adult RPE cells.
Chen et al., 2018:
https://www.sciencedirect.com/science/article/pii/S0945053X17304857

2.1.3.) Biolaminin for photoreceptors

The laminin beta-2 chain is found in the retinal pigmented epithelium and neural neuroepithelium as well as in association with choroidal blood vessels and blood vessels in the neural retina. In both places, it is co-localized with laminin 111 and the culture of human embryonic stem cells on laminin 111 has indeed shown to induce expression of photoreceptor markers (Gong et al., 2008).
Gong et al., 2008:
https://www.sciencedirect.com/science/article/abs/pii/S0014483508001012?via%3Dihub

Libby et al., 2000 showed that the interphotoreceptor matrix contains the laminin a3, a4, a5, b2, b3, g2, and g3 subunits, which suggests the presence of three laminin isoforms: laminin 332, 423 and 523, which may play an important role in photoreceptor development, production, stability and synaptic organization. Due to the importance of the laminin beta-2 chain expression for photoreceptors, LN521 would be a good matrix to start on.
Libby et al., 2000: https://www.jneurosci.org/content/20/17/6517

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2.2.) Liver, Hepatocytes

2.2.1) Biolaminin for hPSC Differentiation into hepatocytes

Takayama et al. 2013 showed that LN111 supported the proliferation of hepatocyte progenitors, derived from human pluripotent stem cells and iPSCs, for more than 3 months (15 passages), and suggested that LN411 and LN511 might promote rather biliary differentiation.
Cameron et al. 2015, showed that the differentiation of human ES cells (hESC) to hepatocytes on human recombinant Biolaminin 521 and Biolaminin 111 substrates significantly improved hepatocyte differentiation, maturation, function, and stabilization of phenotype compared to Matrigel cultured cells. Wang et al. 2017 published a detailed protocol for derivation of hepatocytes from human pluripotent stem cells (hPSC) on LN521 and LN111 (1:3 ratio). Here, highly efficient hepatocyte specification was achieved, with demonstrated improvements in both HLC function and phenotype. Importantly, this system is easy to scale up using research and GMP-grade hPSC lines.
Kanninen et al. 2016 created a xeno-free and chemically defined differentiation protocol with LN511 and LN521 for hPSC and iPSC-derived definitive endoderm (DE) cells and further for hepatic specification.
Vanmarcke et al. compared LN521 to LN511, two different types of recombinant Vitronectin and Matrigel to develop a cost-efficient protocol for differentiation of hPSCs into hepatocyte-like cells (HLCs). LN521 together with an optimized small molecule combination, resulted in HLCs that were transcriptionally identical to HLCs generated using the growth factor combinations.
Ong et al. 2018 developed a screening platform based on the cellular phenotypic characteristics to evaluate the hepatocytes derived from induced pluripotent stem cells (i-Heps) compared to hepatocytes freshly isolated from human liver. LN411 prolonged survival and the functional characteristics of i-Heps.
To generate 3D human livers spheres, Meseguer-Ripolles et al. 2021 (David Hay lab) developed a protocol for the differentiation of hPSCs on LN521 into hepatic progenitors, endothelial cells, and hepatic stellate cells. The combination of all three cell types generates a liver model system that better recapitulates the human liver biology. Ye et al. 2020 described a novel hydrogel based on polyisocyanopeptides (PIC) and laminin-111 for human liver organoid cultures.

Takayama et al. 2013: Long-Term Self-Renewal of Human ES/iPS-Derived Hepatoblast-like Cells on Human Laminin 111-Coated Dishes: Stem Cell Reports
Cameron et al. 2015: Recombinant Laminins Drive the Differentiation and Self-Organization of hESC-Derived Hepatocytes: Stem Cell Reports
Wang et al. 2017: Defined and Scalable Generation of Hepatocyte-like Cells from Human Pluripotent Stem Cells - PMC (nih.gov)
Kanninen et al. 2016: Laminin-511 and laminin-521-based matrices for efficient hepatic specification of human pluripotent stem cells - ScienceDirect
Vanmarcke et al. 2023: Automated Generation of hiPSC-Derived Hepatic Progeny by Cost-Efficient Compounds | Stem Cells | Oxford Academic (oup.com)
Ong et al. 2018: Imaging-Based Screen Identifies Laminin 411 as a Physiologically Relevant Niche Factor with Importance for i-Hep Applications: Stem Cell Reports
Meseguer-Ripolles et al. 2021 (David Hay lab): Protocol for automated production of human stem cell derived liver spheres (nih.gov)
Ye et al. 2020: A Chemically Defined Hydrogel for Human Liver Organoid Culture (nih.gov)

2.2.2.) Biolaminin for fetal hepatocytes

Laminin 411 is a component of the hepatic niche in human fetal liver and Ong et al. 2018 demonstrated improved cell survival of primary fetal hepatocytes on LN411, as compared to collagen.
Ong et al. 2018: Imaging-Based Screen Identifies Laminin 411 as a Physiologically Relevant Niche Factor with Importance for i-Hep Applications: Stem Cell Reports

2.2.3.) Biolaminin for primary human hepatocytes

For human primary hepatocytes in culture the cell viability is maintained by **laminin 521, 111 and 411** (Watanabe et al. 2016, and Ong et al. 2018). Watanabe et al. 2016 demonstrated that the laminin isoforms LN111, LN211, LN221, LN332, LN411, LN421, LN511, and LN521, retained viability, gene expressions, and functional properties of primary human hepatocytes for up to 6 days in culture.
Ong et al. measured protein expression in hepatocytes freshly isolated from normal fetal, neonatal, and adult livers and showed that **LN411** is the relevant laminin isoform for primary hepatocytes. LN411 was found to have the most positive effect on capturing a population of healthy primary adult hepatocytes after cell isolation from biopsy (Fig. 2a).

Watanabe et al. 2016: Maintenance of Hepatic Functions in Primary Human Hepatocytes Cultured on Xeno-Free and Chemical Defined Human Recombinant Laminins - PMC (nih.gov)
Ong et al. 2018: Imaging-Based Screen Identifies Laminin 411 as a Physiologically Relevant Niche Factor with Importance for i-Hep Applications: Stem Cell Reports

2.2.4.) Biolaminin for hepatic progenitors

Takayama et al. 2013 showed that LN-111 supported the proliferation of hepatocyte progenitors, derived from human pluripotent stem cells and iPSCs, for more than 3 months (15 passages), and suggested that LN411 and LN511 might promote rather biliary differentiation.
In comparison, LN521 promoted greater proliferation of Hepatic progenitor cells (HPCs) from rats and increased the maximal cell viability by 2.33-fold, compared to uncoated controls, fibronectin, Matrigel, other laminin isoforms (LN511, LN421, and LN411), or laminin from sarcoma basement membrane, in a study by Ma et al. Inactivation of p53 inhibited the promotion of proliferation by LN-521 and decreased the proliferation of transplanted HPCs *in vivo*.

Takayama et al. 2013: Long-Term Self-Renewal of Human ES/iPS-Derived Hepatoblast-like Cells on Human Laminin 111-Coated Dishes: Stem Cell Reports
Ma et al. 2022: p53 positively regulates the proliferation of hepatic progenitor cells promoted by laminin-521 | Signal Transduction and Targeted Therapy (nature.com)

2.2.4.) Biolaminin for hepatic, 3D liver organoids

To generate 3D human livers spheres, Meseguer-Ripolles et al. 2021 (David Hay lab) developed a protocol for the differentiation of hPSCs on LN521 into hepatic progenitors, endothelial cells, and hepatic stellate cells. The combination of all three cell types generates a liver model system that better recapitulates the human liver biology.
For the combined culture combined hepatocellular carcinoma cell line (HepG2) and hepatic stellate cells (HSCs, 4:1 ratio), Mansouri et al. functionalized fluorinated (PFC) chitosan microparticles (MPs) with LN111, LN511, LN521 or fibronectin on a microfluidic chip. The results showed that cells in assembled liver spheroids responded best to MPs presenting LN511 and LN521.
Ye et al. 2020 described a novel hydrogel based on polyisocyanopeptides (PIC) and LN111 for the expansion and differentiation of human adult stem cell-derived liver organoids. The stem cell phenotype, as well as the proliferation and differentiation capacity of the organoids was maintained in PIC-LN111 organoids over several passages, enabling their subsequent maturation.

Meseguer-Ripolles et al. 2021 (David Hay lab): Protocol for automated production of human stem cell derived liver spheres (nih.gov)
Ye et al. 2020: A Chemically Defined Hydrogel for Human Liver Organoid Culture (nih.gov)
Mansouri et al. 2023: Fabrication of oxygen-carrying microparticles functionalized with liver ECM-proteins to improve phenotypic three-dimensional in vitro liver assembly, function, and responses - Mansouri - 2023 - Biotechnology and Bioengineering - Wiley Online Library

2.3.) Neural

2.3.1.) Biolaminin for dopaminergic neurons

2.3.1.1.) hPSC differentiation into dopaminergic neurons

Parkinson's disease (PD) is a neurodegenerative disorder with loss of dopaminergic neurons in the midbrain (mDA neurons). Zhang at al. tested different Biolaminin® isoforms, LN111, LN411, LN421, LN511, LN332 and LN521, whose mRNA combinations were enriched in ventral midbrain. In comparison, culture of mDA neurons on LN511 increased their numbers 8-fold or 70-fold, compared to LN111 or LN332, respectively. Biolaminin 511 (LN511) promoted the survival and differentiation of mDA neurons, by acting as specific activator of YAP.
Zhang et al., 2017 https://pubmed.ncbi.nlm.nih.gov/28831020/
Nishimura et al. established a protocol with key developmental components (LN511, WNT5A, LXR) for the differentiation of mDA from hESC. Single-cell RNA-sequencing analysis revealed a developmental dynamic similar to that of the endogenous human ventral midbrain and the emergence of high-quality molecularly defined midbrain cell types.
Nishimura et al. 2023: Single-cell transcriptomics reveals correct developmental dynamics and high-quality midbrain cell types by improved hESC differentiation - ScienceDirect

2.3.1.4.) Protocols: hPSC differentiation into dopaminergic neurons

Kirkeby et al. published a protocol were Biolaminin 111 (LN111) was used in the GMP-compatible differentiation of hESCs to dopaminergic neurons. Seven different Biolaminin isoforms were tested, where from four of them (LN111, LN421, LN511, and LN521) efficiently supported adherent differentiation of the ventral mesencephalon (VM) progenitors and LN521 and LN511 selectively supported hPSCs growth. LN111 selectively supported the growth of neural cells but not pluripotent stem cells, achieving a high and reproducible yield of caudalized mesDA VM progenitors.

Kirkeby et al., 2016: Predictive Markers Guide Differentiation to Improve Graft Outcome in Clinical Translation of hESC-Based Therapy for Parkinson's Disease: Cell Stem Cell

The Stem-PD trial is the first cell therapy trial for Parkinson disease in Europe. The good manufacturing practice (GMP)-compliant protocol for the derivation of dopaminergic neurons from human pluripotent cells (hPSC), was realized by using Biolaminin® CT521 and LN521 as cell culture substrates. The recent publication presents quality, safety, and efficacy data supporting the first-in-human STEM-PD phase I/IIa clinical trial along with the trial design.

Kirkeby et al. 2023: Preclinical quality, safety, and efficacy of a human embryonic stem cell-derived product for the treatment of Parkinson's disease, STEM-PD: Cell Stem Cell

Nishimura et al. recently published a step-by-step protocol for the differentiation of hESC into midbrain dopaminergic neurons on LN511.

Nishimura et al. 2023: A protocol for the differentiation of human embryonic stem cells into midbrain dopaminergic neurons - ScienceDirect

2.3.1.3.) Brain organoid - Biosilk

The below listed articles (Fiorenzano et al. 2021 and Sozzi et al. 2022) for brain organoids highlight the advantages of the recombinant spider silk matrix Biosilk and Biolaminins in cultured ventral midbrain organoids. The matrix structure allows an efficient diffusion of media and oxygen, without the risk of necrotic centers, generating highly functional cells even in the core (whole-cell patch clamp). Since Biosilk and Biolaminin are defined and animal-origin-free, the variation between organoids is minimized (scRNA sequencing data), as compared to conventional organoids. Both publications by Fiorenzano et al. 2021 and Sozzi et al. 2022 created ventral midbrain or cerebral 3D organoids, respectively.

Fiorenzano et al. 2021: Single-cell transcriptomics captures features of human midbrain development and dopamine neuron diversity in brain organoids | Nature Communications

Sozzi et al. 2022: https://www.frontiersin.org/articles/10.3389/fcell.2022.1023279/full

The publication by Åstrand et al. 2020 presented results of hPSC cultured on Biosilk (here: FN-silk) coated with LN521 and further differentiated into neuroectodermal tissue. They could show the benefits of the Biosilk pores as media-distributing channels (Fig. 4) and that cells on Biosilk retain self-organizing ability in organoids 60 days after initiation of differentiation (Fig. 6). Additionally, the authors confirmed the functional activity of cells by measuring intracellular calcium oscillations with Fluo-4 (Fig.5-6). Here, the neural organoids are detached from the plate surface after a 48 h (Fig.1) or 21 days (Fig.6) culture period.
Assembly of FN-silk with laminin-521 to integrate hPSCs into a three-dimensional culture for neural differentiation - Biomaterials Science (RSC Publishing) (Åstrand et al. 2020, Biomaterials Science)

Kälvälä et al. investigated the cellular and molecular mechanisms in Parkinson's disease, with a co-culture model of microglia progenitors and midbrain organoid slices utilizing an air-liquid culture system with Biosilk+LN521.
Compared to conventional organoids, the model increased the efficacy and consistency of the integration of microglia progenitors and the microglia progenitors readily differentiated into Iba1+ microglia-like cells with ramified morphology. Additionally, the organoids slices maturated giving rise to astrocytes and oligodendrocyte progenitors and the model significantly improved neuronal functionality in response to stimulation by NMDA, as evaluated by MEA.
Kälvälä et al. 2023: Air-liquid interface culture of midbrain organoids improves neuronal functionality and integration of microglia (biorxiv.org)

2.3.2.) Biolaminin for cortical neurons

We recommend Biolaminin 521 and11 for the culture of hPSC-derived cortical neurons.

Hyvärinen et al. generated electrophysiologically mature neuronal networks from hPSCs on Biolaminin 521, comparing to widely used rat embryonic cortical cultures. Measured by microelectrode arrays (MEA) the hPSC-derived networks developed synchronous activity, with glutamatergic and GABAergic inputs, recapitulating the classical cortical activity for modelling for pharmacological studies and modeling network dysfunctions.

Hyvärinen et al. 2019:Functional characterization of human pluripotent stem cell-derived cortical networks differentiated on laminin-521 substrate: comparison to rat cortical cultures | Scientific Reports (nature.com)

The protocol by Dias et al. described the differentiation of CRISPR/Cas9-edited hiPSC on LN111 into cortical neurons and their identity validation. Cortical neurons expressed increased markers of deep-layer (TBR1 and CTIP2) and upper-layer (CUX1 and BRN2) cortical neurons. This reproducible differentiation protocol resulted in co-cultures of almost pure mature deep-layer and upper-layer neurons, adequately modeling cerebral neural networks.

Dias et al. 2022: Generation of a CHIP isogenic human iPSC-derived cortical neuron model for functional proteomics - ScienceDirect

2.3.3.) Biolaminin for astrocytes

We recommend LN/MX/CT521 or/and LN221/LN211 for the culture of astrocytes. Astrocytes express laminin isoforms 111, 211 and 521 (Sixt et al. 2001; Jucker et al. 1996). Yao et al. 2014 showed that laminin 111 and laminin 211 regulate the differentiation of pericytes and through that maintain the blood-brain barrier integrity. Lundin et al. 2018 generated a protocol culturing human induced pluripotent stem cell (hiPSC)-derived astroglia (NES-Astro) on LN521.
In the publication by Delsing et al. 2019, IPSC-derived Astrocytes, showed a higher expression of several astroglia specific mRNAs and proteins when cultured on LN521, compared to laminin from sarcoma basement membrane.
Izrael et al. published a protocol for good manufacturing practice-grade derivation of astrocyte progenitor cells (APCs) from hESCs on MX/CT521, for transplantation into transgenic mice and rats to evaluate their therapeutic potential. By this the authors demonstrated the safety and potential therapeutic benefits of intrathecal injection of hES-derived astrocytes for the treatment of Amyotrophic lateral sclerosis (ALS).

Sixt et al. 2001: Endothelial Cell Laminin Isoforms, Laminins 8 and 10, Play Decisive Roles in T Cell Recruitment across the Blood–Brain Barrier in Experimental Autoimmune Encephalomyelitis | Journal of Cell Biology | Rockefeller University Press (rupress.org)
Jucker et al. 1996: Laminin alpha 2 is a component of brain capillary basement membrane: reduced expression in dystrophic dy mice - PubMed (nih.gov)
Yao et al. 2014: Astrocytic laminin regulates pericyte differentiation and maintains blood brain barrier integrity | Nature Communications
Lundin et al. 2018: Human iPS-Derived Astroglia from a Stable Neural Precursor State Show Improved Functionality Compared with Conventional Astrocytic Models - PubMed (nih.gov)
Delsing et al. 2019: Enhanced xeno-free differentiation of hiPSC-derived astroglia applied in a blood–brain barrier model (nih.gov)
Izrael et al. 2018: Safety and efficacy of human embryonic stem cell-derived astrocytes following intrathecal transplantation in SOD1G93A and NSG animal models - PubMed (nih.gov)

2.4.) Biolaminin for pancreatic cell culture

We recommend testing LN332, LN411, LN511 and LN521 for the culture of pancreatic-islet cells.
The vascular basement membrane (BM) formed next to the β-cells contains alpha-4 and alpha-5 laminin isoforms (Sigmundsson, 2018; Sixt, 2001). Adult human islets are surrounded by a double BM containing both laminin 511 and laminin 411, but where the BM facing the human endocrine islet is suggested to only contain laminin isoforms 511 and 521 (Virtanen, 2008).
Sigmundsson et al. have developed a novel method to grow and maintain human functional islets on Biolaminin 521. Islets adhered and spread on LN521 to form layers of 1-3 cells in thickness and were functional for at least 14 days in culture, compared to spherical islets in suspension which developed hypoxia and central necrosis within 16 hours. Transplantation mouse islets cultured on α5-laminin-coated polydimethylsiloxane membranes for 3–7 days normalized blood glucose already within 3 days in mice with streptozotocin-induced diabetes.
Molakandov et al. showed the *in vitro* differentiation of hESC into Islet-Like Clusters on MX/CT521 (cell therapy grade Biolaminin 521) and implantation of those clusters into murine diabetes models. Clusters enriched with cells expressing the selective marker CD49A, improved their therapeutic activity and reduced the number of cells needed to normalize glycemia.
Sigmundsson et al. 2018: Culturing functional pancreatic islets on α5-laminins and curative transplantation to diabetic mice - ScienceDirect
Molakandov et al. 2021: Frontiers | Selection for CD26− and CD49A+ Cells From Pluripotent Stem Cells-Derived Islet-Like Clusters Improves Therapeutic Activity in Diabetic Mice (frontiersin.org)

Nikolova et al. from Dr. Doug Melton's group showed that laminin isoforms LN411 and LN511 stimulate β1 integrin signaling–dependent insulin production in vitro. The authors suggest that treating islets with these biologically relevant laminin isoforms prior to transplantation could help maintain insulin production until new capillaries are formed in transplanted islets.
Nikolova et al. 2006: The vascular basement membrane: a niche for insulin gene expression and Beta cell proliferation - PubMed (nih.gov)

Parnaud et al. established that β cells cultured on LN332 spread out and secreted twice more insulin in response to glucose compared to cells cultured on plastic, poly-L-lysine. Armanet et al., showed that laminin 332 plays a physiologically relevant role in human islets since laminin 332 is expressed and secreted by human islets. After transplantation, LN411-induced insulin-producing cells-derived cells markedly improved the symptoms and survival of type-1 diabetic rats.
Parnaud, 2006: Blockade of beta1 integrin-laminin-5 interaction affects spreading and insulin secretion of rat beta-cells attached on extracellular matrix - PubMed (nih.gov)
Armanet, 2009: Regulated laminin-332 expression in human islets of Langerhans - PubMed (nih.gov)

Qu et al. showed that LN411 can act as a potent differentiation inducer of UC-MSC into insulin-producing cells, by increasing expression of pancreatic precursor markers and markedly up-regulate insulin expression, both at the mRNA and protein level.
Qu et al. 2014: Laminin 411 acts as a potent inducer of umbilical cord mesenchymal stem cell differentiation into insulin-producing cells | Journal of Translational Medicine | Full Text (biomedcentral.com)

2.5.) Biolaminin for human pluripotent stem cell (hPSC)

1) Human Embryonic stem cells - hESC (key LN521 papers)

Laminin 521 and laminin 511 are expressed around the stem cells in the inner cell mass and support survival and self-renewal of the pluripotent stem cells. On LN521 human embryonic stem (hES) and induced pluripotent stem (iPS) cells grow as a monolayer, providing homogenous, high-quality cell culture that can be passaged at very low cell densities (Rodin et al. 2014). hES cells cultured on LN521 exhibited less differentiation, faster cell growth and attachment when compared to hES cells cultured on LN121 or Matrigel. The results indicate a positive effect of LN521 in stabilizing pluripotency gene expression (Albalushi et al. 2017). The process of dynamic blebbing, occurring when dissociated single hESC are dissociated into single cells or small colonies, was significantly inhibited by LN521 and cell attachment was accelerated and significantly fewer apoptotic cells were observed, with hES cells plated on LN521, compared to Matrigel (Weng et al. 2018).

Rodin et al. 2014 a: Clonal culturing of human embryonic stem cells on laminin-521/E-cadherin matrix in defined and xeno-free environment | Nature Communications
Rodin et al. 2014 b: Monolayer culturing and cloning of human pluripotent stem cells on laminin-521–based matrices under xeno-free and chemically defined conditions | Nature Protocols
Albalushi et al. 2017: Laminin 521 Stabilizes the Pluripotency Expression Pattern of Human Embryonic Stem Cells Initially Derived on Feeder Cells - PMC (nih.gov)
Weng et al: 2018: Dynamic blebbing: A bottleneck to human embryonic stem cell culture that can be overcome by Laminin-Integrin signaling - ScienceDirect

2.5.1.) Biolaminin for human induced pluripotent stem cells - hiPSC

Lu et al described a chemically defined culture system based on xeno-free media and LN521 substrate, which supported efficient reprogramming of normal or diseased skin fibroblasts from human of different ages into hiPSCs with a 15e30 fold increase in efficiency over conventional methods. The derived iPSCs maintained long-term self-renewal, normal karyotype and pluripotency LN521 supported the robust derivation of human induced pluripotent stem (hiPS) cells was by using non-integrating Sendai virus (SeV) vector mediated reprogramming of dermal fibroblasts (Uhlin et al. 2014). Lotila et al. 2022 reported that LN521 supports the generation of a human induced pluripotent stem cell (hiPSC) line from peripheral blood mononuclear cells of a multiple sclerosis patient. Xu et al. showed efficient reprogramming of dermal fibroblasts into hiPSC on MX/CT521.
See defined culture of fibroblast under serum-free conditions here: **Error! Reference source not found. Error! Reference source not found.**.

Uhlin et al. 2017: Integration Free Derivation of Human Induced Pluripotent Stem Cells Using Laminin 521 Matrix | Protocol (jove.com)
Lu et al. 2014: A defined xeno-free and feeder-free culture system for the derivation, expansion and direct differentiation of transgene-free patient-specific induced pluripotent stem cells - PubMed (nih.gov)
Lotila et al. 2022:Establishment of a human induced pluripotent stem cell line (TAUi008-A) derived from a multiple sclerosis patient - ScienceDirect
Xu et al. 2022: Multiple therapeutic effects of human neural stem cells derived from induced pluripotent stem cells in a rat model of post-traumatic syringomyelia - eBioMedicine (thelancet.com)

2.5.2.) Biolaminin for generation of iPSC lines incl. genetic testing and trilineage differentiation capacity

Schuster et al. and Vasylovska et al. generated several iPSC cell lines on Biolaminin 521. Kuebler et al. established the Spanish iPSC haplobank on Biolaminin 521 from CD34+ cells of 7 donors.

Vasylovska et al. 2021: Generation of human induced pluripotent stem cell (iPSC) lines (UUMCBi001-A, UUMCBi002-A) from two healthy donors - ScienceDirect
Kuebler et al. 2023: Generation of a bank of clinical-grade, HLA-homozygous iPSC lines with high coverage of the Spanish population | Stem Cell Research & Therapy | Full Text (biomedcentral.com)

2.5.3.) Biolaminin for single cell passaging

Biolaminin 521 was shown to increase single cell cloning efficiency compared to commonly used ECMs such as Matrigel or Geltrex (Vallone et al. 2020).
For CRISPR/Cas9 protocols on hESC, LN521 coating was supporting the single cells seeding at a very low density (Kim et al. 2017 and Carlson-Stevermer et al. 2015).
Biolaminin 521 can support cell survival at a seeding density as low as 5,000 cells/cm^2.

Vallone et al. 2020: Methods for Automated Single Cell Isolation and Sub-Cloning of Human Pluripotent Stem Cells - Vallone - 2020 - Current Protocols in Stem Cell Biology - Wiley Online Library
Kim et al. 2017: Generation of a Nrf2 homozygous knockout human embryonic stem cell line using CRISPR/Cas9 - ScienceDirect
Carlson-Stevermer et al. 2015): High-Content Analysis of CRISPR-Cas9 Gene-Edited Human Embryonic Stem Cells - PMC (nih.gov)

2.6.) Biolaminin for mouse pluripotent stem cells (mPSC)

For mouse embryonic stem cells, LN511 alone, without the addition of leukemia inhibitory factor (LIF) or other differentiation inhibitors, is sufficient to enable self-renewal of mouse embryonic stem cells (mESCs) for up to 169 days (31 passages) with a maintained expression of pluripotency markers (Domogatskaya et al., 2008). The reason is that Laminin-511 is the only laminin isoform expressed in the early mouse embryo and crucial for the acquisition of naive pluripotency (Boroviak et al., 2014). The binding of mESC is likely to occur through a3B1 and a6B1 integrin receptors expressed on the mES cell surface (Lecht et al., 2013).

Laminins are highly evolutionary conserved proteins. Therefore, studies done in other species can also give valuable information for the other species.

Domogatskaya et al., 2008: Laminin-511 but Not -332, -111, or -411 Enables Mouse Embryonic Stem Cell Self-Renewal In Vitro | Stem Cells | Oxford Academic (oup.com)
Boroviak et al., 2014: The ability of inner-cell-mass cells to self-renew as embryonic stem cells is acquired following epiblast specification | Nature Cell Biology
Lecht et al., 2013: Enhanced reseeding of decellularized rodent lungs with mouse embryonic stem cells - PMC (nih.gov)

2.6.) Biolaminin for skin cell culture

For the culture of skin-related carcinomas the laminin isoforms **LN332**, **LN511** and **LN521** can be recommended.

Publication evidence has been collected on the increased expression of laminin **332** in squamous cell carcinoma (SCC) associating with poor prognosis.
Caley et al. generated specific knockdowns of laminin 332 subunits in cutaneous SCC cell lines, herby showing that that loss of the laminin α3 chain but not of β3 or γ2 chains correlates with poorly differentiated tumours in.vivo and increases cell invasiveness through activation of the ROCK (rho-associated protein kinase) signalling pathway. Additionally, the loss of the laminin α3 chain activates the reprogramming of the immune microenvironment.
Caley et al. 2020: Loss of the laminin subunit alpha-3 induces cell invasion and macrophage infiltration in cutaneous squamous cell carcinoma* | British Journal of Dermatology | Oxford Academic (oup.com)

Oikawa et al. found the expression of laminin-111, laminin-332, laminin-511 and laminin-521, but not laminin-211 and laminin-411, strongly promoted cell migration in melanoma specimens and melanoma cell lines indicated. The authors conclude that, the α5 (corresponding to **LN511** and **LN521**) laminins are the isoforms most relevant to melanoma cell migration.
Oikawa et al. 2011: Melanoma cells produce multiple laminin isoforms and strongly migrate on α5 laminin(s) via several integrin receptors – ScienceDirect