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Selected Papers for Tissue Specific Laminin Applications

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hESC/iPSC/genome editing Applications

Clonal culturing of human embryonic stem cells on laminin-521/E-cadherin matrix in defined and xeno-free environment
Rodin S., Antonsson L., Niaudet C., Simonson O.E., Salmela E., Hansson E.M., Domogatskaya A., Xiao Z., Damdimopoulou P., Sheikhi M., Inzunza J., Nilsson A.S., Baker D., Kuiper R., Sun Y., Blennow E., Nordenskjöld M., Grinnemo K.H., Kere J., Betsholtz C., Hovatta O., Tryggvason K.
Nature Communications, 2014
Clonal derivation and single-cell expansion of hPSCs on laminin-521.This article provides scientific evidence that LN-521 is the optimal matrix for generation and culture of human pluripotent stem cells. It is described in detail how this physiologically relevant laminin establishes genetically stable hESC lines in an efficient, defined, xeno-free and feeder-free procedure, suitable for stem cell banking and regenerative medicine applications. It is even possible to derive embryonic stem cells from a single blastomere, thereby avoiding the ethical dilemma associated with the destruction of donated embryos.

Monolayer culturing and cloning of human pluripotent stem cells on laminin-521 based matrices under xeno-free and chemically defined conditions
Rodin S., Antonsson L., Hovatta O., Tryggvason K.
Nature Protocols, 2014
Detailed step-by-step protocols for transfer, expansion and clonal growth of hPSCs on laminin-521. Here the authors describe predictable monolayer, xeno-free and defined culturing of hPSCs on LN-521. In the article there is an important assembly of protocols for LN-521 based hPSC bulk expansion, true clone generation, the secure transfer step-by-step from feeders to LN-521, freezing and thawing as single cells using FREEZEstem. There are also critical steps and reagents included for easier handling of more difficult lines and a useful trouble shooting guide for solving problems faster.

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
Lu H.F., Chai C., Lim T.C., Leong M.F., Lim J.K., Gao S., Lim K.L., Wan A.C.
Biomaterials, 2014
Reprogramming of iPSCs on LN-521 and direct differentiation to dopaminergic cells on Laminin-521.This article demonstrates LN-521 as an optimal defined, xeno- and feeder-free matrix for the reprogramming of human iPS cells. Laminin-521 achieves high-efficiency reprogramming in different media, fast and easy expansion as well as direct differentiation to dopaminergic neurons on LN-521. The authors conclude that the efficient transgene-free hiPSC derivation and expansion on LN-521 enables clinical applications useful for human patient iPSCs and derivatives for cellular therapy.

A Novel In Vitro Method for Detecting Undifferentiated Human Pluripotent Stem Cells as Impurities in Cell Therapy Products Using a Highly Efficient Culture System
Tano K., Yasuda S., Kuroda T., Saito H., Umezawa A., Sato Y.
PLOS ONE, 2014
In the article the authors use LN-521 for a safety step for iPS cells going for therapeutic purpose. This group is responsible for dictating the safety aspects of future regen med in Japan. Tano and colleagues show a novel approach based on LN-521 for direct and sensitive detection of trace amounts of residual undifferentiated hPSCs for cell therapy products. The presence of contaminating hPSCs in cell therapy products is a major quality concern associated with tumorigenicity and this first in vitro assay is direct, simple and cost-effective. The highly efficient culture system using LN-521 detected colony forming hPSCs spiked into primary human MSCs or neurons at a ratio as low as 0.001%–0.01%.

Optimization of slow cooling cryopreservation for human pluripotent stem cells
Miyazaki T., Nakatsuji N., and Suemori H.
Genesis, 2013

Increased viability of hPSCs through single-cell freezing/thawing/expansion on Laminin-521.This is one of the first customer publications that demonstrates Laminin-521 as an optimal xeno- and feeder-free matrix for pluripotent stem cells. The authors show cells should be cryopreserved as single cells for highest survival which is specifically supported by Laminin-521 that promotes adhesion and self-renewal of fully dissociated single cells in the absence of ROCK inhibitor. They demonstrate 80-90% survival of hPSCs post-thawing and 60% survival following subculture on Laminin-521, allowing for efficient and easy handling of cells and bulk storage of high-quality hPSCs.

a-5 Laminin Synthesized by Human Pluripotent Stem Cells Promotes Self-Renewal
Laperle A., Hsiao C., Lampe M., Mortier J., Saha K., Palecek S.P., and Masters K.S.
Stem Cell Reports, 2015
The authors study the role of endogenously produced extracellular matrix (ECM) components in regulating hPSC fates. They identify a-5 laminin as a signature ECM component endogenously synthesized by undifferentiated hESC and hiPSC cultured on defined substrates. The cells also produced collagen I but no vitronectin or fibronectin. Knockdown and disruption of the LAMA5 gene dramatically reduced hPSC self-renewal and increased apoptosis without affecting the expression of pluripotency markers. Self-renewal and survival was restored to wild-type levels by culturing the LAMA5-deficient cells on exogenous laminin-521. Synthemax or Vitronectin could not restore survival. Treatment of LAMA5-deficient cells with blebbistatin or a ROCK inhibitor partially restored self-renewal and diminished apoptosis. These results demonstrate that endogenous a-5 laminin promotes hPSC survival and self-renewal in an autocrine and paracrine manner. A good publication that also show how much better laminin-521 performs compared to other competitor matrices.

Higher-Density Culture in Human Embryonic Stem Cells Results in DNA Damage and Genome Instability
Jacobs K., Zambelli F., Mertzanidou A., Smolders I., Geens M., Nguyen H.T., Barbé L., Sermon K., Spits C.
Stem Cell Reports, 2016
Here, the authors demonstrate a direct correlation between medium acidification linked to culture density, and the occurrence of DNA damage and genomic alterations in hESC grown on feeder layers. This, in turn, results in an increase of cells in G1 and a stalling of the S phase, without an increase in cell death or a loss of pluripotency. The DNA effects are rapid and occurs in the short time span of a single passage. However, culture density has no effect on the level of apoptosis. Increasing the frequency of the medium refreshments minimizes the levels of DNA damage and genetic instability. hESC grown on laminin-521 show a decreased proneness to acquiring DNA damage during suboptimal culture conditions, such as medium acidification during high culture density.

Generation of human iPS cell line CTL07-II from human fibroblasts, under defined and xeno-free conditions
Kele M., Day K., Rönnholm H., Schuster J., Dahl N., Falk A.
Stem cell research, 2016
CTL07-II is a healthy feeder-free and characterized human induced pluripotent stem (iPS) cell line cultured under xeno-free and defined conditions. iPS cell coating during derivation and expansion was human recombinant Laminin-521The line is generated from healthy human fibroblasts with non-integrating Sendai virus vectors encoding the four Yamanaka factors, OCT4, SOX2, KLF4 and cMYC. The generated iPS cells are free from reprogramming vectors and their purity, karyotypic stability and pluripotent capacity is confirmed.

Directed differentiation of human iPSC into insulin producing cells is improved by induced expression of PDX1 and NKX6.1 factors in IPC progenitors
Walczak M. P., Drozd A. M., Stoczynska-Fidelus E., Rieske P., Grzela D.P.
Journal of Translational Medicine, 2016
Here, the authors show that the highest efficiencies of reprogramming of fibroblasts were obtained on Laminin-511 and Laminin-521, compared to other coatings.

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MSC Applications

CD49f Acts as an Inflammation Sensor to Regulate Differentiation, Adhesion and Migration of Human Mesenchymal Stem Cells
Yang Z., Dong P., Fu X., Li Q., Ma S., Wu D., Kang N., Liu X., Yan L., Xiao R.
Stem Cells, 2015
Here, we studied the role of CD49f (also known as integrin α6) in bone marrow MSCs. CD49f is preferentially expressed in fetal cells rather than adult cells, CD49f-positive BM-MSCs possess higher CFU-F formation ability and differentiation potential than CD49f negative cells, and the CD49f expression of BM-MSCs gradually decreases during in vitro passaging. An adhesion assay showed strong adhesion of BM-MSCs to both laminin 511 and 521 that were significantly higher than the control group coated with BSA, and the adhesion occurred evenly throughout the well. Pre-blocking of CD49f on BM-MSCs inhibited the adhesion of fetal BM-MSCs to laminin 511 and 521. Also, CD49f knockdown dramatically decreased the differentiation of BMSCs. Inflammation (TNF-a) down-regulated CD49f in BMSCs with impaired differentiation, decreased adhesion to laminins and increased migration. This study provide evidence for CD49f as a stemness marker of BMSCs which is correlated with cell adhesion on laminin-521 and -511.

Laminin-521 Promotes Rat Bone Marrow Mesenchymal Stem Cell Sheet Formation on Light-induced Cell Sheet Technology
Jiang Z., Xi Y., Lai K., Wang Y., Wang H., Yang G.
BioMed Research International, 2016
The cell sheet technology is an area of research that is of great interest for tissue engineering and regenerative medicine. Here, the authors investigated the effects of an ECM coating on rat bone marrow mesenchymal stem cells (rBMSC) cultured on light-induced TiO2 nanodot films. Cell sheets can be detached on a TiO2 nanodot-coated quartz substrate by using UV365 illumination. The effects of rat fibronectin, human recombinant laminin-521, -511, -421, and -111 on the formation of cell sheets were investigated and also compared to uncoated films. The result showed the highest success for laminin-521. rBMSCs rapidly attached and spread on films coated with laminin-521 (1.2 ug/ml) and formed intact cell sheets after 5 days of culture. Laminin-521 promote the formation of rBMSC sheets with good viability under hyperconfluent conditions (4 to 8 layers of cells). The cells also maintained multilineage potential, including osteogenic, adipogenic, and chondrogenic differentiation. The cell sheets formed had rich ECM (including collagen I) and cells were connected with each other in a dense network-like tissue. rBMSC cultured on uncoated surface and cells partially detached and failed to form cell sheets. In summary, laminin-521 and UV365 illumination systems provided a simple, rapid, and effective cell sheets strategy.

Bone Marrow Mesenchymal Stem Cells Adhesion Assay
Yang Z. and Xiao R.
Bio-protocol, 2016
Here, the authors present a protocol for culture of bone marrow MSC (BM-MSCs) on laminin-521 or laminin-511. The protocol is based on the method by Siler et al., 2000, and can easily be translated to MSCs from other origin or alternative ECMs coating. Both laminin isoforms show a significantly better efficient attachment compared to uncoated wells and also support seeding of a lower cell number compared to uncoated plats. The BM-MSCs adhere to the laminin-coated wells within 10 min, while for non-coated wells, it may take longer time. In this protocol, a final coating concentration of 2 μg/cm^2 is used but can effectively be lowered 4-10 times without loss of function.

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HSC Applications

Characterization of bone marrow laminins and identification of alpha5-containing laminins as adhesive proteins for multipotent hematopoietic FDCP-Mix cells.
Gu Y., Sorokin L., Durbeej M., Hjalt T., Jonsson J.I., Ekblom M.
Blood, 1999
Laminins interact in vitro with mature blood cells and malignant hematopoietic cells. Here they show that laminins are widely expressed in mouse bone marrow. Laminin a2, a4, and α5 polypeptides were found suggesting presence of laminin-211, laminin-411, and laminin-511 in bone marrow. Gene expression of laminin a1, a2, a4, and α5 chains in long-term bone marrow cultures, indicating up-regulation of laminin a1 expression in vitro. Laminins containing α5 chain, in contrast to laminin-1, were strongly adhesive for multipotent hematopoietic FDCP mix cells. Integrin a6 and b1 chains mediated this adhesion.

Characterization and functional analysis of laminin isoforms in human bone marrow.
Siler U., Seiffert M., Puch S., Richards A., Torok-Storb B., Müller C.A, Sorokin L., Klein G.
Blood, 2000
Based on gene expression, laminin-411/421 and laminin-511/521 are the most abundant laminin isoforms synthesized by human bone marrow stromal cells. Laminin-511/521 preparations showed strong adhesive interactions with human $CD34^+$ cell lines. Antibodies against the B1 integrin subunit inhibited these interactions. In addition to its adhesion-mediating properties, laminin-511/521 preparations also showed a mitogenic activity for human hematopoietic progenitor cells. Other laminin isoforms tested, especially laminin-111 and laminin-211 and 221, showed only weak or no adhesive interactions with the hematopoietic cell lines tested and are suggested to play a minor role in the hematopoietic microenvironment. In the bone marrow, LN-511 and 521 are the major laminins and show strongly adhesive and mitogenic activities toward early developing HSCs.

Contribution of α6 integrins to hematopoietic stem and progenitor cell homing to bone marrow and collaboration with α4 integrins
Qian H., Tryggvason K., Jacobsen S.E., Ekblom M.
Blood, 2006
Integrin a6 chain is ubiquitously expressed in human and mouse hematopoietic stem and progenitor cells. Laminin-411 and -511, are present in subendothelial basement membranes of sinusoids in bone marrow, at sites of hematopoietic cell development and trafficking and might therefore regulate HSC functions. In this paper they show that mouse HSC and progenitors express a6B1 integrin which mediates high cell adhesion to laminin-511 and 521 and to laminin-411 to a lower extent. Blocking of a6 significantly reduced progenitor cell homing to bone marrow in mice. Integrin a4 receptors are also important for homing of HSCs to bone marrow (but not to spleen). The first data showing that a6 integrins (LN521/511 binding) function in vivo as hematopoietic stem and progenitor cell homing receptors.

Laminin isoform–specific promotion of adhesion and migration of human bone marrow progenitor cells
Gu Y-C., Kortesmaa J., Tryggvason K., Persson J., Ekblom P., Jacobsen S-E., Ekblom M.
HEMATOPOIESIS, 2003
Here they studied human bone marrow cell adhesion to laminin-511/521, laminin-411, laminin-111, and fibronectin. About 35% to 40% of $CD34^+$ and $CD34^+CD38^-$ stem and progenitor cells adhered to laminin-511/521, and 45% to 50% adhered to fibronectin, whereas they adhered less to laminin-411 and laminin-111. Adhesion of $CD34^+CD38^-$ cells to laminin-511/521 was maximal without integrin activation, whereas adhesion to other proteins was dependent on protein kinase C activation. Integrin a6 chain expressed on most CD34+ and CD34+CD38-cells. Laminin-511/521 was highly adhesive to lineage-committed myelomonocytic and erythroid progenitor cells and most lymphoid and myeloid cell lines studied, whereas fibronectin and laminin-411 was less adhesive. Laminin-511/521was a ubiquitous adhesive protein for differentiated precursors of both B-lymphocytic, erythroid, megakaryocytic, and myelomonocytic cell lineages, whereas adhesion to laminin-411 and laminin-111 was restricted to a few cell lines. $CD34^+$ cell migration was greatly enhanced through membranes coated with Laminin-511/521 and laminin-411.

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Endothelial Applications

VASCULAR ENDOTHELIA AND BLOOD

Integrin α6β1 Is the Main Receptor for Vascular Laminins and Plays a Role in Platelet Adhesion, Activation, and Arterial Thrombosis
Schaff M., Tang CJ., Maurer E., Bourdon C., Receveur N., Eckly A., Hechler B., Arnold C., de Arcangelis A., Nieswandt B., Denis C.V., Lefebvre O., Georges-Labouesse E., Gachet C., Lanza F., Mangin P.H
Circulation, 2013
Show that laminin-411, laminin-511 and laminin-521, but not laminin-211, allow efficient platelet adhesion and activation across a wide range of arterial wall shear rates. Adhesion was critically dependent on integrin α6β1 and the glycoprotein Ib-IX complex, which binds to plasmatic von Willebrand factor adsorbed on laminins. Glycoprotein VI did not participate in the adhesive process but mediated platelet activation induced by α5-containing laminins. Platelet-specific knockout of integrin α6 failed to adhere to laminin-411, laminin-511, and laminin-521 but responded normally to a series of agonists. α6β1-Deficient mice presented a marked decrease in arterial thrombosis in 3 models of injury of the carotid, aorta, and mesenteric arterioles. The tail bleeding time and blood loss remained unaltered, indicating normal hemostasis. This study reveals an unsuspected important contribution of laminins to thrombus formation in vivo and suggests that targeting their main receptor, integrin α6β1, could represent an alternative antithrombotic strategy with a potentially low bleeding risk.

Differentiation of Human Embryonic Stem Cells to Endothelial Progenitor Cells on Laminins in Defined and Xeno-free Systems
Nguyen M.T.X., Okina E., Chai X., Tan K.H., Hovatta O., Ghosh S., Tryggvason K.
Stem Cell Reports, 2016
Here, the authors developed a chemically defined, xeno-free protocol for differentiation of hESCs to endothelial progenitor cells (EPCs) using LN521 as the main culture substrate. The EPCs derived were functional and expressed both progenitor and mature endothelial markers. They were able to generate about 95% functional EPCs defined as VEGFR2+CD34+CD31+VE-Cadherin+. RNA-sequencing analyses of hESCs, EPCs, and primary human umbilical vein endothelial cells showed differentiation-related EC expression signatures, regarding basement membrane composition, cell-matrix interactions, and changes in endothelial lineage markers. Six-week continuous culturing allows the hESC derived EPSs to mature further, relative to HUVECs. These results may facilitate production of stable ECs for the treatment of vascular diseases and in vitro cell modeling.

CORNEAL ENDOTHELIA

Laminin-511 and -521 Enable Efficient In Vitro Expansion of Human Corneal Endothelial Cells (HCEC)
Okumura N., Kakutani K., Numata R., Nakahara M., Schlötzer-Schrehardt U., Kruse F., Kinoshita S., Koizumi N.
IVOS Cornea, 2015
Laminin-511 and -521 were expressed in Descemet's membrane and corneal endothelium. These laminin isoforms significantly enhanced the in vitro adhesion and proliferation, and differentiation of HCECs compared to uncoated control, fibronectin and collagen I. iMatrix also supported HCEC cultivation with a similar efficacy to that obtained with full-length laminin. Functional blocking of a3b1 and a6b1 integrins suppressed the adhesion of HCECs even in the presence of laminin-511.

Identification and Potential Application of Human Corneal Endothelial Progenitor Cells
Hara S., Hayashi R., Soma T., Kageyama T., Duncan T., Tsujikawa M., Nishida K.
Stem Cells Dev., 2014
This article demonstrates for the first time that Laminin-511 is an optimal, human matrix for the isolation and expansion of corneal endothelial progenitors. The authors show that the proliferative capacity of these endothelial progenitors is very high on Laminin-511 compared to conventional methods. Laminin-511 can be used to rapidly isolate and expand a homogenous population of an endothelial progenitor cells that can be differentiated to endothelial cells in a biorelevant environment. The authors demonstrate that the proliferative capacity of these endothelial progenitors is very high on Laminin-511 compared to conventional methods. Laminin-511 can thus be

used to rapidly isolate and expand a homogenous population of endothelial progenitors that can be differentiated to endothelial cells in a biorelevant environment. Main points of the article are: 1) High proliferative capacity in serum-free media compared to standard methods, 2) Large numbers of cells generated, 3) Facilitates rapid isolation of a homogenous population of endothelial progenitors, 4) Enables differentiation to endothelial cells in a biorelevant environment, 5) Cells can be subcultured for at least 5 passages

The Different Binding Properties of Cultured Human Corneal Endothelial Cell Subpopulations to Descemet's Membrane Components
Toda M, Ueno M, Yamada J, Hiraga A, Tanaka H, Schlötzer-Schrehardt U, Sotozono C, Kinoshita S, Hamuro J
Invest Ophthalmol Vis Sci. 2016
In this study, the authors examined the binding ability of cHCECs subpopulations to major Descemet's membrane components that distribute to the endothelial face; that is, laminin-511, -411, Type-IV collagen, and proteoglycans. Each subpopulation was prepared by controlling the culture conditions or by using magnetic cell separation, and then confirmed by staining with several cell-surface markers. Binding abilities of HCEC subpopulations were examined by adding the cells to culture plates immobilized with collagens, laminins, or proteoglycans, and then centrifuging the plates. The cHCECs showed **best attachment to laminin laminin-521 and -511**. The cells showed a weaker binding to laminin-411, laminin-332, Type-IV collagen. The minimum concentrations necessary for the observed cell binding in this study were as follows: laminin-521 and -511, 3 ng/mL; laminin-411, 2.85 ug/mL; Type-IV collagen, 250 ng/mL. Cells suspended in Opti-MEM-I or Opeguard-MA were bound to laminin, yet no binding was observed in cells suspended in BSS-Plus. Both the fully differentiated, mature cHCEC subpopulations and the epithelial-to-mesenchymal– transitioned (EMT)-phenotype subpopulation were found to attach to laminin- or collagen-coated plates. Interestingly, the binding properties to laminins differed among those subpopulations. Although the level of cells adhered to the laminin-411–coated plate was the same among the cHCEC subpopulations, the fully differentiated, mature cHCEC subpopulations was significantly more tightly bound to laminin-511 than was the EMT-phenotype subpopulations. These findings suggest that the binding ability of cHCECs to major Descemet's membrane components is distinct among cHCEC subpopulations, and that Opti-MEM-I and Opeguard-MA are useful cell-suspension vehicles for cell-injection therapy. This research group focused on developing a novel medical approach, termed cell-injection therapy, for the treatment of patients with endothelial dysfunction.

BBB ENDOTHELIA

Endothelial Cell Laminin Isoforms, Laminins 8 and 10, Play Decisive Roles in T Cell Recruitment Across the Blood–Brain Barrier in Experimental Autoimmune Encephalomyelitis
Sixt M., Engelhardt B., Pausch F., Hallmann R., Wendler O., Sorokin L.M
J Cell Biol., 2001
Laminin-411 and laminin-511 are described as the major laminin isoforms in vascular basement membranes. Their expression was influenced by pro-inflammatory cytokines or angiostatic agents. Inflammatory cuffs occurred exclusively around endothelial basement membranes containing laminin-411, whereas in the presence of laminin-511 no infiltration was detectable. Integrin α6 and β-dystroglycan were prominent in CNS blood vessels, whereas no staining was observed for integrin α3, α7, and β4 subunits. One of the major laminin receptors, integrin α6β1, was localized predominantly on the endothelial cells, where it is likely to mediate interactions with the endothelial cell laminin-411 and 511, whereas astrocyte endfeet appear to utilize a different receptor for interactions with the parenchymal laminins. β-Dystroglycan occurred predominantly on astrocyte endfeet.

The extracellular matrix protein laminin-10 promotes blood–brain barrier repair after hypoxia and inflammation in vitro
Kangwantas K., Pinteaux E., Penny J.
Journal of neuroinflammation 2016
Integrity of the BBB is primarily maintained by brain endothelial cells, the tight junctions between them and their attachment to the blood vessel basement membrane (mainly composed fibronectin, collagen IV, and laminin-411 and -511). Here the authors used an in vitro model of the BBB, composed of primary rat brain endothelial cells grown on these different ECM proteins. The in vitro BBB model was exposed to oxygen-glucose deprivation with or without re-oxygenation, and in the absence or the presence of IL-1β in order to mimic the ischemic and inflammatory conditions that occur during stroke. They show that LN-511 plays a key role in maintenance of BBB integrity and that it's a key ECM molecule involved in BBB repair after hypoxic injury and inflammation. The brain endothelial cells did not adhere well to LN-411.

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Epithelial Applications

INTESTINE

Abnormal Wnt and PI3Kinase Signaling in the Malformed Intestine of lama5 Deficient Mice
Ritié L., Spenlé C., Lacroute J.I., Bolcato-Bellemin A-L., Lefebvre O., Bole-Feysot C., Jost B., Klein A., Arnold C., Kedinger M., Bagnard D., Orend G., Simon-Assmann P.
PLOS ONE, 2012
Laminin-511 is highly expressed in the intestine. To understand the mechanistic role of laminin-511 in tissue homeostasis, the researchers used RNA profiling of embryonic intestinal tissue of lama5 knockout mice and identified a lama5 specific gene expression signature. They show that laminin α5 plays a crucial role in both epithelial and mesenchymal (smooth muscle) cell behavior by inhibiting Wnt and activating PI3K signaling. We conclude that conflicting signals are elicited in the absence of lama5, which alter cell adhesion, migration as well as epithelial and muscle differentiation. The LMa5 deficient intestine also displays a smooth muscle defect and myogenic differentiation markers are affected. Laminin-511 supports adhesion of epithelial cells and Akt phosphorylation. Laminin-511 stimulates spreading of epithelial and muscle cells (compared to laminin-111). Inhibition of Akt with wortmannin abolished spreading of epithelial cells on laminin-511 as evidenced by cell laminin-511 specifically activates Akt through the PI3K pathway in intestinal epithelial but not in mesenchymal cells. Cell migration was also higher on Laminin-511. Laminin-511 also protects cells against H2O2-induced apoptosis.

Laminin α5 influences the architecture of the mouse small intestinal mucosa
Mahoney Z.X., Stappenbeck T.S., Miner J.H
J Cell Sci. 2008
The villus basement membrane is rich in laminin α5. Here the authors show that diminution of laminin α5 in a mouse model led to a compensatory deposition of colonic laminins that resulted in a transformation from a small intestinal to a colonic mucosal architecture. The alteration in mucosal architecture was associated with reduced levels of nuclear p27Kip1, a cell cycle regulator, and altered intestinal epithelial cell proliferation, migration, and differentiation. The results suggest that laminin α5 plays a crucial role in establishing and maintaining the specific mucosal pattern of the mouse small intestine.

Developmental Expression and Cellular Origin of the Laminin a2, a4, and α5 Chains in the Intestine
Lefebvre O., Sorokin L., Kedinger M., Simon-Assmann P.
Developmental Biology, 1999
In this study, the authors examine the expression patterns and the cellular origins of the laminin a2, a4, and α5 chains in the developing mouse intestine and in in vitro mouse/chick or chick/mouse interspecies hybrid intestines. All three laminin alpha-chains are highest in the fetal intestine undergoing intense morphogenetic movements. Laminin a4 are associated with mesenchyme-derived cell populations such as endothelium and smooth muscle. In contrast, laminin a2 and α5 chains participate in the structural organization of the subepithelial basement membrane and, in the mature intestine, show a complementary pattern of expression. All three laminins chains occur in the smooth muscle basement membrane. Laminin a2 was found to be deposited into the basement membrane exclusively by mesenchymal cells, and the laminin α5 chain was deposited by both epithelial and mesenchymal cells in an apparently developmentally regulated pattern.

SKIN

Polymerized Laminin-332 Matrix Supports Rapid and Tight Adhesion of Keratinocytes, Suppressing Cell Migration
Kariya Y., Sato H., Katou N., Kariya Y., Miyazaki K.
PLOS ONE, 2012
Laminin-332 is known to supports the stable anchoring of basal keratinocytes to the epidermal basement membrane but is also motility factor for wound healing and cancer invasion. Here they investigated Laminin-332 matrices deposited by normal human keratinocytes and several cancer cell lines. All types of the cells efficiently

deposited Laminin-332 on the culture plates in specific patterns. On the contrary, laminins containing laminin ß1 and/or c1 chains (such as Lm511 and Lm311) were not deposited on the culture plates even if secreted into culture medium. The deposited Laminin-332 matrix showed a mesh-like network structure as analyzed by electron microscopy, suggesting that Lm332 was highly polymerized. Laminin-332 matrix rather suppressed the migration of keratinocytes as compared with purified Lm332 (not a BioLamina product), which highly promoted the cell migration. The Lm332 matrix supported adhesion of keratinocytes much more strongly and stably than purified Lm332. Integrin a3ß1 bound to the Lm332 matrix at a three times higher level than purified Lm332. These results indicate that the polymerized Lm332 matrix supports stable cell adhesion whereas unassembled soluble Lm332 supports cell migration. The question is though how the purified Ln-332 looked like. Difficult to purify and might be fractionated.

Laminin 10/11: an alternative adhesive ligand for epidermal keratinocytes with a functional role in promoting proliferation and migration.
Pouliot N., Saunders N.A., Kaur P.
Exp Dermatol. 2002
The authors investigated the expression and function of laminin-511 and -521 in neonatal and adult human skin. They found that the laminin-α5 chain is expressed abundantly in the basement membrane underlying the interfollicular epidermis and the blood vessels in the dermis. Interestingly, while the expression level of the well-studied laminin-5 isoform did not change significantly with age, laminin-511 and -521 appeared to decrease in the basement membrane underlying the epidermis, in adult skin. In contrast, the levels of laminin-511 and -521 in the basement membrane underlying blood vessels remained unchanged in neonatal vs. adult skin.

HAIR

Laminin-10 is crucial for hair morphogenesis.
Li J., Tzu J., Chen Y., Zhang Y.P., Nguyen N.T., Gao J., Bradley M., Keene D.R., Oro A.E., Miner J.H., Marinkovich M.P.
EMBO J. 2003
Here, the authors describe the essential role of laminin-511 in hair follicle development. Treatment of human scalp xenografts with antibodies to laminin-10, or its receptor β1 integrin, produced alopecia. Skin from lama5-null mice fails to support hair development. Absence of laminin-511 in transgenic mice led to a number of developmental abnormalities, including arrest of hair development and deficient Shh expression in hair follicles. Transplantation of skin grafts from these *Lama5*-null mouse embryos onto healthy mice failed to support hair growth. Intriguingly, purified laminin-511 (isolated from A549 lung carcinoma cell conditioned medium) was able to restore hair development in the *Lama5*-null skin. The authors conclude that laminin-511 is required for hair follicle development and report the first use of exogenous protein to correct a cutaneous developmental defect.

Spatial and temporal control of laminin-332 (5) and -511 (10) expression during induction of anagen hair growth.
Sugawara K., Tsuruta D., Kobayashi H., Ikeda K., Hopkinson S.B., Jones J.C., Ishii M.
J Histochem Cytochem. 2007
Here they characterized changes in laminin isoform expression during hair cycling. At the mRNA level, laminin-511 expression undergo a steady increase during anagen stages. In contrast, laminin-332 expression is initially upregulated in outer root sheath (ORS) keratinocytes at anagen II and then transiently downregulated. Immunohistochemistry demonstrated that laminin-332 and α6β4 integrin were well co-localized, but their signals were remarkably decreased in the lower half of follicles after anagen VI. In hair follicle culture, laminin-511/521-rich human placental laminin enhanced hair growth, whereas recombinant laminin-332 antagonized hair growth induced by laminin-511. Our results indicate a positive role for laminin-511 and a negative role for laminin-332 on hair growth.

Laminin-511 is an epithelial message promoting dermal papilla development and function during early hair morphogenesis.
Gao J., DeRouen M.C., Chen C.H., Nguyen M., Nguyen N.T., Ido H., Harada K., Sekiguchi K., Morgan B.A., Miner J.H., Oro A.E., Marinkovich M.P.
Genes Dev. 2008.
Here the authors demonstrate the mechanism of how laminin-511 controls hair morphogenesis. Dermal papilla (DP) from laminin-511 mutants showed developmental defects by E16.5 in mice, including a failure to maintain

expression of the key morphogen noggin. Laminin-511 mutant DP showed decreased length and structure of primary cilia in vitro and in vivo. Laminin-511, but not laminin-111, restored primary cilia formation in lama5−/− mesenchyme and triggered noggin expression in an Shh- and PDGF-dependent manner. Hair development required the B1 integrin binding but not the heparin binding domain of laminin-511. Inhibition of laminin-511 receptor B1 integrin disrupted DP primary cilia formation as well as hair development. These studies show that epithelial-derived laminin-511 is a critical early signal that directs ciliary function and DP maintenance as a requirement for hair follicle down-growth.

Laminin 10/11- an alternative adhesive ligand for epidermal keratinocytes with a functional role in promoting proliferation and migration
Pouliot N., Saunders N.A., Kaur P.
Experimental Dermatology, 2002
They investigated the expression and function of the laminin-511 and -521 in neonatal and adult human skin. These isoforms are expressed abundantly in the basement membrane underlying the inter-follicular epidermis and the blood vessels in the dermis. The expression levels did not change significantly with age, but appeared to decrease in the basement membrane underlying the epidermis, in adult skin. In contrast, the levels of laminin-511 and -521 in the basement membrane underlying blood vessels remained unchanged in neonatal vs. adult skin. An *in vitro* cell adhesion assays demonstrated that laminin-511 and -521 is potent adhesive substrates for both neonatal and adult keratinocytes and that this adhesion is mediated by the a3b1 and a6b4 integrins. Further, laminin-511 and -521 provide a proliferative signal for neonatal foreskin keratinocytes, adult breast skin keratinocytes, and even a human papillomavirus type-18 transformed tumorigenic keratinocyte cell line *in vitro*. Laminin-511 and -521 also stimulate keratinocyte migration in an *in vitro* wound healing assay. These results provide strong evidence for a functional role for laminin-10/11 in epidermal proliferation during homeostasis, wound healing and neoplasia.

Integrin-linked kinase regulates the niche of quiescent epidermal stem cells.
Morgner J., Ghatak S., Jakobi T., Dieterich C., Aumailley M., Wickström S.A.
Nat Commun., 2015
In the present study the authors conclude that the precise ratio between LN-332 and LN-511 adjusts activities of key signalling pathways that determine SC activation within the hair follicle bulge niche. They suggest that integrin-linked kinase (ILK) is required for the maintenance of quiescent bulge SCs through remodelling of the ECM niche, thereby governing the activation and maintenance of hair follicle stem cells (HFSCs). ILK mediates deposition of inverse laminin-332 and -511 gradients within the basement membrane (BM) wrapping the hair follicles. The precise ratio of LN-511 and LN-332 regulates core SC fate-determining signalling pathways and that this ratio is disturbed in the absence of ILK, leading to aberrant SC activation and failure to re-establish quiescence. The BM composition tunes activities of Wnt and transforming growth factor-β pathways and subsequently regulates HFSC activation. LN-511, present at low levels around the bulge and at higher levels around the hair germ/TACs, promotes Tgf-β signalling, whereas LN-332, highly expressed along the IFE and to a lesser extent along the upper regions of HFs, suppresses Wnt/β-catenin signalling. Notably, reconstituting an optimal laminin microenvironment restores the altered signalling in ILK-deficient cells.

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Pancreatic Applications

Laminin 411 acts as a potent inducer of umbilical cord mesenchymal stem cell differentiation into insulin-producing cells
Qu H., Liu X., Ni Y., Jiang Y., Feng X., Xiao J., Guo Y., Kong D., Li A., Li X., Zhuang X., Wang Z., Wang Y., Chang Y., Chen S., Kong F., Zhang X., Zhao S., Sun Y., Xu D., Wang D., Zheng C.
Journal of Translational Medicine, 2014
Efficient induction of differentiation to insulin-producing cells from MSCs. Up-regulated insulin expression at both mRNA and protein levels. Administration of the insulin producing cells in T1 diabetes rats rapidly 1) down-regulated fasting blood glucose levels, 2) significantly reduced the HbA1c concentration and 3) markedly improved the symptoms and survival of the rats.

Co-culture with extracellular matrix proteins reduces hypoxia-induced human islet cell death
Brandhorst et al., Xenotransplantation, Abstracts of the IPITA-IXA-CTS 2015 Joint Congress November 15–19, 2015, Melbourne, Australia
Islets are experiencing hypoxic conditions after transplantation. The aim of this study was to assess the effect of collagen IV and laminin isoform -521, -511 and -411 on survival and function of isolated human islets exposed to severe hypoxia. Compared with hypoxic controls (100%) all ECMs significantly increased islet recovery after culture at 0.75% oxygen ranging from 163 +/- 12% to 173 +/- 28% ($P < 0.05$) using collagen IV or laminin-411, respectively. Increased post-culture recovery correlated with decreased islet fragmentation which was lowest using laminin-521 (66%, $P < 0.01$) and laminin-511 (66% $P < 0.05$). Islet ROS generation was also lowest after culture with laminin-521 and laminin-511. Islet viability was increased in all experimental groups when compared to controls but was highest using collagen IV and laminin-511. This observation corresponds to the insulin response after glucose challenge that was best preserved when collagen IV or laminin-511 were used for islet incubation.

The Vascular Basement Membrane: A Niche for Insulin Gene Expression and B Cell Proliferation
Nikolova G., Jabs N., Konstantinova I., Domogatskaya A., Tryggvason K., Sorokin L., Fässler R., Gu G., Gerber H-P., Ferrara N., Melton D.A., Lammert E.
Developmental Cell, 2006
Mouse pancreatic islets intimately interact with endothelial cells and differentiation and delamination of insulin-producing b cells from pancreatic epithelium strictly require endothelial cells. Doug Melton and colleagues show that BMs within islets is formed and found exclusively around capillaries but not islet cells. Islet endothelial cells express laminin a4 and α5. Laminins promote insulin gene expression and proliferation in B-cells and B1-intergrin is required for this laminin response. Laminin-411 and -511 worked well but also laminin-111 which shows that the applied laminin does not necessarily have to be endothelial cell-derived. Research on islet transplantation has shown that it takes about 1–2 weeks for transplanted islets to become revascularized in the host and the authors suggest that treating islets with these laminins prior to transplantation will help maintain insulin production until new capillaries are formed in transplanted islets.

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Cardiac Applications

A Chemical Probe that Labels Human Pluripotent Stem Cells
Hirata N., Nakagawa M., Fujibayashi Y., Yamauchi K., Murata A., Minami I., Tomioka M., Kondo T., Kuo T-F., Endo H., Inoue H., Sato S., Ando S., Kawazoe Y., Aiba K., Nagata K., Kawase E., Chang Y-T., Suemori H., Eto K., Nakauchi H., Yamanaka S., Nakatsuji N., Ueda K., Uesugi M.
Cell Reports, 2014
The Yamanaka group uses cardiac specific laminin-211 as the matrix to differentiate iPS cells to cardiomyocytes in a biorelevant environment specific to heart cells. This thus shows that you can use laminin-211 as a cardiac matrix. 326 fluorescent compounds screened to identify a fluorescent probe that is selective for human pluripotent stem cells compared to differentiated cells. hiPSCs were cultured on 3.5 cm culture dishes coated with human laminin 211 and cardiac differentiation was carried out. Cardiac colonies were harvested on day 15 and cultured for 7–10 days in floating culture. A majority of the prepared cells expressed the cardiac markers: cardiac troponin T, a-actinin, and NKX2.5.

A Small Molecule that Promotes Cardiac Differentiation of Human Pluripotent Stem Cells under Defined, Cytokine- and Xeno-free Conditions
Minami I., Yamada K., Otsuji T.G., Yamamoto T., Shen Y., Otsuka S., Kadota S., Morone N., Barve M., Asai Y., Tenkova-Heuser T., Heuser J.E., Uesugi M., Aiba K., and Nakatsuji N.
Cell Reports, 2012
In this study, they report of a small molecule that promote cardiac differentiation of hPSCs. By using this chemical, a xeno-free and cytokine-free cardiac differentiation protocol was achieved. In a part of the study cardiac differentiation on surface coating with gelatin or human laminin-211. Cell attachment on gelatin or laminin-211 is essential for efficient differentiation, suggesting that mechanotransduction or integrin signaling from interaction with these substrates might be important. iPSCs were differentiated into cardiac colonies on human laminin 211-coated dishes in cytokine-free and xeno-free defined medium containing HAS.

Chemically defined generation of human cardiomyocytes
Burridge P., Elena Matsa E., Shukla P., Lin Z., Churko J., Ebert A., Lan F., Diecke S., Huber B., Mordwinkin N., Plews J., Abilez O., Cui B., Gold J. & Wu J.
Nature methods, 2014
Cardiac differentiation strategy using a chemically defined medium consisting of just three components: the basal medium RPMI1640, l-ascorbic acid 2-phosphate and rice-derived recombinant human albumin. this protocol produced contractile sheets of up to 95% TNNT2+ cardiomyocytes at a yield of up to 100 cardiomyocytes for every input pluripotent cell. They first assessed chemically defined pluripotent culture on other defined matrices: rh E-cadherin, rh vitronectin, laminin-521, iMatrix-511, human fibronectin and a fibronectin mimetic. Laminin-based matrices resulted in higher growth rates compared to the vitronectin peptide. Fibronectin-based matrices did not support pluripotent growth. All five suitable matrices supported efficient differentiation in CDM3 but only the laminin-based matrices maintained long-term adhesion (>15 d) during CDM3 cardiac differentiation. The authors still performed all subsequent characterization on the vitronectin peptide since they think that the laminin matrices are prohibitively expensive for large-scale application.

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Hepatic Applications

Long-Term Self-Renewal of Human ES/iPS-Derived Hepatoblast-like Cells on Human Laminin 111-Coated Dishes
Takayama K., Nagamoto Y., Mimura N., Tashiro K., Sakurai F., Tachibana M., Hayakawa T., Kawabata K., Mizuguchi H.
Cell Stem Cell Reports, 2013
The authors of this important study demonstrated that laminin-111 is the optimal matrix for hepatoblasts. In addition to solving the need of efficiently expanding and purifying this liver progenitor, the matrix provides an important safety mechanism as LN-111 selectively only maintain hepatoblasts while eliminating residual undifferentiated cells that cannot survive and self-renew on laminin-111.

CCAAT/enhancer binding protein-mediated regulation of TGFβ receptor 2 expression determines the hepatoblast fate decision
Takayama K., Kawabata K., Nagamoto Y., Inamura M., Ohashi K., Okuno H., Yamaguchi T., Tashiro K., Sakurai F., Hayakawa T., Okano T., Furue M.K. and Mizuguchi H.
Development, 2014
Examined the function of TGFBR2 in the hepatoblast fate decision using hESC-derived HBC. hESC-derived HBCs purified and maintained (HBCs passaged more than three times) on human laminin 111 (LN111)-coated dishes were used. The HBC population were nearly homogeneous and expressed human hepatoblast markers such as alpha-fetoprotein (AFP), albumin (ALB), cytokeratin 19 (CK19) and EPCAM, and most of the colonies observed on human LN111-coated plates were ALB and CK19 double positive. The HBCs were capable of repopulating the liver of carbon tetrachloride (CCl4)-treated immunodeficient mice. This study reveals a molecular mechanism underlying the lineage commitment of human hepatoblasts (hepatocyte and biliary differentiation) controlled by a gradient of TGFβ signaling. It provides the first evidence of c/EBP-mediated regulation of TGFBR2 expression in the human hepatoblast fate decision.

Recombinant Laminins Drive the Differentiation and Self-Organization of hESC-Derived Hepatocytes
Cameron K., Tan R., Schmidt-Heck W., Campos G., Lyall M.J, Wang Y., Lucendo-Villarin B., Szkolnicka D., Bates N., Kimber S.J., Hengstler J.G., Godoy P., Forbes S.J., Hay D.C.
Stem Cell Reports, 2015
Human ES cells cultured on human recombinant laminin-521 and laminin-111 show efficient hepatocyte specification, maturation, function and stabilization of phenotype. The results presented in the paper represents a significant advance compared to any previous published data especially regarding metabolic activity and functional organization. Cells cultured on the laminin matrices exhibited significantly increased metabolic function relative to cells on Matrigel and human primary hepatocytes. The P450 enzyme activities are likely linked to the differences in cell organization observed. The laminin cultured hepatocyte-like cells were arranged in lobule like structures, reminiscent of regenerating liver, with positive staining for MRP1 and MRP2 and were capable of biliary efflux.

Laminin 411 and 511 promote the cholangiocyte differentiation of human induced pluripotent stem cells
Takayama K., Mitani S., Nagamoto Y., Sakurai F., Tachibana M., Taniguchi Y., Sekiguchi K., Mizuguchi H.
Biochem Biophys Res Commun. 2016
Here the authors searched for a suitable extracellular matrix to promote cholangiocyte differentiation from human iPS cells, and found that both laminin 411 and laminin 511 were suitable for this purpose. Differentiated human iPSC to cholangiocyte-like cells via hepatoblasts-like cells (derived according to previous papers). The hepatoblast-like cells were mixed in a collagen gel and plated and for cholangiocyte differentiation, laminin was added to the medium. The gene expression levels of the cholangiocyte markers, aquaporin 1 (AQP1), SRY-box 9 (SOX9), cystic fibrosis transmembrane conductance regulator (CFTR), G protein-coupled bile acid receptor 1 (GPBAR1), Jagged 1 (JAG1), secretin receptor (SCTR), and g-glutamyl transferase (GGT1) were increased by using laminin 411 or laminin 511 as a matrix. In addition, the percentage of AQP1-positive cells was increased from 61.8% to 92.5% by using laminin 411 or laminin 511. Furthermore, the diameter and number of cysts consisted of cholangiocyte-like cells were increased when using either matrix.

Laminin-511 and laminin-521 based matrices for efficient hepatic specification of human pluripotent stem cells

Kanninen L.K., Harjumäki R., Peltoniemi P., Bogacheva M.S., Salmi T., Porola P., Niklander J., Smutny T., Urtti A., Yliperttula M.L., Lou Y-R.

Biomaterials, 2016

In this study the authors used laminin-511, laminin-521, and fibronectin, as culture matrices for hPSC-derived definitive endoderm cells. By screening the acellular matrix produced by HepaRG cells and found that laminin-511 (LN-511), laminin-521 (LN-521), and fibronectin were highly expressed. The authors observed that laminin-511 and laminin-521 either alone or in combination support the hepatic specification. They did not observe any improvement in the cell differentiation efficacy with fibronectin. The expression of the laminin-511/521-specific integrins increased during the definitive endoderm induction and hepatic specification. The hepatic cells differentiated on laminin matrices showed upregulation of liver-specific markers both at mRNA and protein levels, secreted human albumin, stored glycogen, and exhibited cytochrome P450 enzyme activity and inducibility. Use of recombinant matrix proteins is faster, more consistent, more efficient, and more scalable compared to HepaRG-derived acellular matrix.

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Renal Applications

Glomerular endothelial cells and podocytes jointly synthesize laminin-1 and -11 chains
St John P.L. and Abrahamson D.R
Kidney International, 2001
During the glomerular basement membrane assembly, laminin-111 is initially expressed in vascular clefts of comma-a dn S-shaped bodies and is eventually replaced by laminin-521 which persists into maturation. Post-fixation immunoelectron microscopy of developing mouse and 2-3 days old mice kidney was performed and showed Intracellular labeling for laminin-111 and laminin-521 in developing glomerular endothelial cells and podocytes. In early capillary loop stage GBMs laminin-111 was absent but the expression of α5 was strong (developmental switch). The results show that both endothelial cells and podocytes synthesize laminin but highest level of laminin synthesis by endothelial cells.

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Intestinal Applications

Laminin α5 influences the architecture of the mouse small intestinal mucosa
Mahoney Z.X., Stappenbeck T.S., Miner J.H
J Cell Sci. 2008
The villus basement membrane is rich in laminin α5. Here the authors show that diminution of laminin α5 in a mouse model led to a compensatory deposition of colonic laminins that resulted in a transformation from a small intestinal to a colonic mucosal architecture. The alteration in mucosal architecture was associated with reduced levels of nuclear p27Kip1, a cell cycle regulator, and altered intestinal epithelial cell proliferation, migration, and differentiation. The results suggest that laminin α5 plays a crucial role in establishing and maintaining the specific mucosal pattern of the mouse small intestine.

Abnormal Wnt and PI3Kinase Signaling in the Malformed Intestine of lama5 Deficient Mice
Ritié L., Spenlé C., Lacroute J.I., Bolcato-Bellemin A-L., Lefebvre O., Bole-Feysot C., Jost B., Klein A., Arnold C., Kedinger M., Bagnard D., Orend G., Simon-Assmann P.
PLOS ONE, 2012
Laminin-511 is highly expressed in the intestine. To understand the mechanistic role of laminin-511 in tissue homeostasis, the researchers used RNA profiling of embryonic intestinal tissue of lama5 knockout mice and identified a lama5 specific gene expression signature. They show that laminin α5 plays a crucial role in both epithelial and mesenchymal (smooth muscle) cell behavior by inhibiting Wnt and activating PI3K signaling. We conclude that conflicting signals are elicited in the absence of lama5, which alter cell adhesion, migration as well as epithelial and muscle differentiation. The LMα5 deficient intestine also displays a smooth muscle defect and myogenic differentiation markers are affected. Laminin-511 supports adhesion of epithelial cells and Akt phosphorylation. Laminin-511 stimulates spreading of epithelial and muscle cells (compared to laminin-111). Inhibition of Akt with wortmannin abolished spreading of epithelial cells on laminin-511 as evidenced by cell laminin-511 specifically activates Akt through the PI3K pathway in intestinal epithelial but not in mesenchymal cells. Cell migration was also higher on Laminin-511. Laminin-511 also protects cells against H2O2-induced apoptosis.

Designer matrices for intestinal stem cell and organoid culture
Gjorevski N., Sachs N., Manfrin A., Giger S., Bragina M.E., Ordóñez-Morán P., Clevers H., Lutolf M.P.
Nature letters, 2016
Here the authors used modular synthetic hydrogel (crosslinked poly (ethylene glycol) (PEG)) to define the key extracellular matrix (ECM) parameters that govern intestinal stem cell (ISC) expansion and organoid formation, and show that separate stages of the process require different mechanical environments and ECM components. Fibronectin-based adhesion was sufficient for ISC survival and proliferation and high matrix stiffness significantly enhanced ISC expansion through a yes-associated protein 1 (YAP)-dependent mechanism. ISC differentiation and organoid formation, on the other hand, required a soft matrix and full-length laminin-111-based adhesion. The authors also produced mechanically dynamic matrices that were initially optimal for ISC expansion and subsequently permissive to differentiation and intestinal organoid formation.

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Myogenic Applications

Laminin 521 maintains differentiation potential of mouse and human satellite cell-derived myoblasts during long-term culture expansion
Penton C.M., Badarinarayana V., Prisco J., Powers E., Pincus M., Allen R.E., August P.R.
Skeletal Muscle, 2016
Here, the authors comprehensively examine the effect of physiologically relevant laminins, laminin-211 and laminin-521, compared to traditionally utilized ECMs (e.g., laminin 111, gelatin, and Matrigel) to assess their capacity to propagate and preserve myogenic differentiation potential. The results demonstrate laminin-521 is a superior substrate for both short-term and long-term myogenic cell culture applications compared to other commonly utilized substrates. Laminin-521 also provides more consistent and reliable differentiation over long-term culture. Laminin-521 supported increased proliferation in early phases of expansion and was the only substrate facilitating high-level fusion following eight passages in mouse myoblast cell cultures. In human myoblast cell cultures, laminin 521 supported increased proliferation during expansion and superior differentiation with myotube hypertrophy. Counterintuitively however, laminin 211, the native laminin isoform in resting skeletal muscle, resulted in low proliferation and poor differentiation in mouse and human cultures. Matrigel performed well in short-term mouse studies but showed high amounts of variability following long-term expansion.

Laminin α5 chain is required for intestinal smooth muscle development
Bolcato-Bellemin A-L., Lefebvre O., Arnold C., Sorokin L., Miner J. H., Kedinger M., Simon-Assmann P.
Developmental Biology, 2003
Here, the function of the laminin α5 chain in the developing intestine was defined by analyzing laminin α5 -/- mutants and by grafting experiments. The authors show that laminin α5 plays a major role in smooth muscle organization and differentiation, as excessive folding of intestinal loops and delay in the expression of specific markers are observed in laminin α5 -/- mice. Loss of α5 expression was paralleled by ectopic or accelerated deposition of laminin a2 and a4 chains; this may explain why no obvious defects were observed in the villous form and enterocytic differentiation. Lack of the laminin α5 chain was accompanied by a decrease in epithelial a3B1 integrin receptor expression adjacent to the epithelial basement membrane and of Lutheran blood group glycoprotein in the smooth muscle cells, indicating that these receptors are likely mediating the α5 interactions. Taken together, the laminin α5 chain is essential for normal development of the intestinal smooth muscle

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Neural Applications

The adhesion GPCR GPR126 has distinct, domain-dependent functions in Schwann cell development mediated by interaction with Laminin-211
Petersen S.C., Luo R., Liebscher I., Giera S., Jeong S-J., Mogha A., Ghidinelli M., Feltri M.L., Schöneberg T., Piao X., Monk K.R.
Neuron, 2015
The authors demonstrate that the binding of Laminin-211 to the GPR126 either suppress or promote the activation GPR126-CTF and is laminin-211 concentration dependent. Schwann cell myelination starts with activation of the GPR126 which results in cleavage into two fragments, NTF and CTF. NTF initiates radial, axonal sorting and CTF promotes the axonal wrapping thus driving the terminal differentiation. The study beautifully shows the that laminin-211 is a fundamental regulator of Schwann cell biology and that its actual binding to the GPR126 regulate radial sorting and myelination. Laminin-211 as a novel ligand for GPR126 that differentially modulates receptor signaling to control both early and late Schwann cell development. GPR126 is an adhesion GPCR required for Schwann cell myelination.
supports the morphogenetic recapitulation of cortical development.

The extracellular matrix protein laminin-10 promotes blood–brain barrier repair after hypoxia and inflammation in vitro
Kangwantas K., Pinteaux E., Penny J.
Journal of neuroinflammation 2016
Integrity of the BBB is primarily maintained by brain endothelial cells, the tight junctions between them and their attachment to the blood vessel basement membrane (mainly composed fibronectin, collagen IV, and laminin-411 and -511). Here the authors used an in vitro model of the BBB, composed of primary rat brain endothelial cells grown on these different ECM proteins. The in vitro BBB model was exposed to oxygen-glucose deprivation with or without reoxygenation, and in the absence or the presence of IL-1β in order to mimic the ischemic and inflammatory conditions that occur during stroke. They show that LN-511 plays a key role in maintenance of BBB integrity and that it's a key ECM molecule involved in BBB repair after hypoxic injury and inflammation.

Neurons From Human Pluripotent Stem Cells Under Xeno-Free Conditions Restore Motor Deficits in Parkinsonian Rodents
Niclis J.C., Gantner C.W., Alsanie W.F., McDougall S.J., Bye C.R., Elefanty A.G., Stanley E.G., Haynes J.M., Pouton C.W., Thompson L.H., Parish C.L.
Stem cells translational medicine, 2016
In this study, the authors describe the first fully defined feeder- and xenogeneic-free protocol for the generation of vmDA neurons from hPSCs. The protocol is translational across multiple embryonic and induced hPSC lines. hPSCs were cultured xeno-free on laminin-521 in TeSR2. For vmDA differentiation, two xeno-free matrix proteins, vitronectin and human laminin-521, were compared for their ability to replace Matrigel. Both matrices facilitated appropriate patterning, however, only laminin-521 supported the long-term attachment of neural precursors. This "next generation" protocol consistently increases both the yield and proportion of vmDA neural progenitors (OTX2/FOXA2/LMX1A) and neurons (FOXA2/TH/PITX3) that display classical vmDA metabolic and electrophysiological properties. The mechanism underlying these improvements are identified and demonstrate clinical applicability with the first report of scalability and cryopreservation of bona fide vmDA progenitors at a time amenable to transplantation. Finally, transplantation of xeno-free vmDA progenitors from LMX1A- and PITX3-eGFP reporter lines into Parkinsonian rodents demonstrates improved engraftment outcomes and restoration of motor deficits.

Predictive Markers Guide Differentiation to Improve Graft Outcome in Clinical Translation of hESC-Based Therapy for Parkinson's Disease
Kirkeby A., Nolbrant S., Tiklova K., Heuer A., Kee N., Cardoso T., Rylander Ottosson D., Lelos M.J., Rifes P., Dunnett S.B., Grealish S., Perlmann T., Parmar M.
Cell Stem Cell, 2016
Here, the authors developed a good manufacturing practice (GMP) differentiation protocol for highly efficient and

reproducible production of transplantable dopamine progenitors from hESCs on laminin-111. They identified predictive markers expressed in dopamine neuron progenitors that correlate with graft outcome in an animal model of Parkinson's disease. Timed FGF8b resulted in high yield of caudal VM cells and good graft outcome correlate with markers of caudal VM and MHB. Commonly used markers did not accurately predict in vivo subtype-specific maturation. Instead, we identified a specific set of markers associated with the caudal midbrain that correlate with high dopaminergic yield after transplantation in vivo. Using these markers, a GMP-adapted dopamine differentiation protocol was developed.

Ablation of astrocytic laminin impairs vascular smooth muscle cell function and leads to hemorrhagic stroke
Chen Z-L., Yao Y., Norris E.H., Kruyer A., Jno-Charles O., Akhmerov A., Strickland S.
J. Cell Biol. 2013
Astrocytes express laminin-111 and 211 and assemble basement membranes (BMs) at their endfeet. Here the authors show that ablation of astrocytic laminin disrupted endfeet BMs and led to hemorrhage in deep brain regions of adult mice. The lack of astrocytic laminin led to impaired function of vascular smooth muscle cells, fragmentation and vascular wall disassembly where astrocytes have a closer association with VSMCs in small arterioles. Acute disruption of astrocytic laminin in the striatum of adult mice also impaired vascular smooth muscle cells function, indicating that laminin is necessary for vascular smooth muscle cells maintenance. In vitro, both astrocytes and astrocytic laminin promoted brain vascular smooth muscle cells differentiation.

YAP and TAZ control peripheral myelination and the expression of laminin receptors in Schwann cells
Poitelon Y., Lopez-Anido C., Catignas K., Berti C., Palmisano M., Williamson C., Ameroso D., Abiko K., Hwang Y., Gregorieff A., Wrana JL., Asmani M., Zhao R., Sim FJ., Wrabetz L., Svaren J., Feltri ML.
Nature Neuroscience, 2016
A mechanistic article adding further evidence for the importance of laminin-211 for radial sorting and proper axon myelination by Schwann cells. The authors show that laminin-211 in combination with mechanical stimuli activate and modulate Yap and Taz, that are downstream effectors in the Hippo pathway, required for radial sorting for axons and subsequent myelination.

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Eye Applications

RETINA:

Xeno-Free and Defined Human Embryonic Stem Cell-Derived Retinal Pigment Epithelial Cells Functionally Integrate in a Large-Eyed Preclinical Model
Plaza Reyes A., Petrus-Reurer S., Antonsson L., Stenfelt S., Bartuma H., Panula S., Mader T., Douagi I., Andre H., Hovatta O., Lanner F., Kvanta A.
Stem Cell Reports, 2015
A publication by the groups of Drs. Hovatta, Lanner and Kvanta describe production of hESC-RPE cells in a xeno-free and defined manner. In the paper they describe an effective differentiation methodology using human recombinant laminin-521 matrix with a xeno-free and defined medium. The differentiated RPE cells exhibit native characteristics including morphology, pigmentation, marker expression, monolayer integrity, polarization and phagocytic activity. The authors also established a large-eyed geographic atrophy model that allowed *in vivo* imaging of the hESC-RPE and host retina. Cells were transplanted in suspension and showed long-term integration and formed polarized monolayers exhibiting phagocytic and photoreceptor rescue capacity.

Retinal Pigment Epithelial Cells Synthesize Laminins, Including Laminin 5, and Adhere to Them through a3- and a6-Containing Integrins
Aisenbrey S., Zhang M., Bacher D., Yee J., Brunken W.J., Hunter D.D.
Invest Ophthalmol Vis Sci., 2006
The multilayered extracellular matrix underlying the retina is Bruch's membrane (BM). Here they show that BM contains laminin chains that could form laminin-111, -332, -511, and -521. RPE cells synthesized these laminin chains in vitro, hence, RPE cells may synthesize BM laminins. The RPE cells adhered to the BM component collagen IV, but preferentially adhered to laminins. Of the laminins tested, the RPE cells adhered preferentially to laminin 332. The RPE cells interacted with these laminins via a3 and a6 containing integrins.

Laminin Expression in Adult and Developing Retinae: Evidence of Two Novel CNS Laminins Libby R.T., Champliaud M-F, Claudepierre T., Xu Y., Gibbons E.P., Koch M., Burgeson R.E., Hunter D.D, Brunken W.J.
The Journal of Neuroscience, 2000
Here, they examine the expression of all known laminin chains within the retina. The inter-photoreceptor matrix (and, during early development, the subretinal space) contains the laminin a3, a4, α5, b2, b3, g2, and g3 chains. This suggests the presence of three laminins: laminin-332, laminin-423 and laminin-523. These laminin isoforms could exert important effects on photoreceptor development and may play a role in photoreceptor production, stability and synaptic organization.

CORNEA:

Laminin-511 and -521 Enable Efficient In Vitro Expansion of Human Corneal Endothelial Cells (HCEC)
Okumura N., Kakutani K., Numata R., Nakahara M., Schlötzer-Schrehardt U., Kruse F., Kinoshita S., Koizumi N.
IVOS Cornea, 2015
Laminin-511 and -521 were expressed in Descemet's membrane and corneal endothelium. These laminin isoforms significantly enhanced the in vitro adhesion and proliferation, and differentiation of HCECs compared to uncoated control, fibronectin and collagen I. iMatrix also supported HCEC cultivation with a similar efficacy to that obtained with full-length laminin. Functional blocking of a3b1 and a6b1 integrins suppressed the adhesion of HCECs even in the presence of laminin-511.

Identification and Potential Application of Human Corneal Endothelial Progenitor Cells
Hara S., Hayashi R., Soma T., Kageyama T., Duncan T., Tsujikawa M., Nishida K.
Stem Cells Dev. 2014
This article demonstrates for the first time that Laminin-511 is an optimal, human matrix for the isolation and expansion of corneal endothelial progenitors. The authors show that the proliferative capacity of these endothelial progenitors is very high on Laminin-511 compared to conventional methods. Laminin-511 can be used to rapidly

isolate and expand a homogenous population of a endothelial progenitor cells that can be differentiated to endothelial cells in a biorelevant environment. The authors demonstrate that the proliferative capacity of these endothelial progenitors is very high on Laminin-511 compared to conventional methods. Laminin-511 can thus be used to rapidly isolate and expand a homogenous population of endothelial progenitors that can be differentiated to endothelial cells in a biorelevant environment. Main points of the article are: 1) High proliferative capacity in serum-free media compared to standard methods, 2) Large numbers of cells generated, 3)Facilitates rapid isolation of a homogenous population of endothelial progenitors, 4)Enables differentiation to endothelial cells in a biorelevant environment, 5) Cells can be subcultured for at least 5 passages

The Different Binding Properties of Cultured Human Corneal Endothelial Cell Subpopulations to Descemet's Membrane Components
Toda M., Ueno M., Yamada J., Hiraga A., Tanaka H., Schlötzer-Schrehardt U., Sotozono C., Kinoshita S., Hamuro J.
Invest Ophthalmol Vis Sci. 2016
In culture, human corneal endothelial cell (cHCEC) tend to enter into cell-state transition (CST), such as epithelial-to-mesenchymal transition (EMT) or fibrosis, thus resulting in the production of different subpopulations. In this study, the authors examined the binding ability of cHCECs subpopulations to major Descemet's membrane components that distribute to the endothelial face; that is, laminin-511, -411, Type-IV collagen, and proteoglycans. Each subpopulation was prepared by controlling the culture conditions or by using magnetic cell separation, and then confirmed by staining with several cell-surface markers. Binding abilities of HCEC subpopulations were examined by adding the cells to culture plates immobilized with collagens, laminins, or proteoglycans, and then centrifuging the plates. The cHCECs showed **best attachment to laminin laminin-521 and -511**. The cells showed a weaker binding to laminin-411, laminin-332, Type-IV collagen. The minimum concentrations necessary for the observed cell binding in this study were as follows: laminin-521 and -511, 3 ng/mL; laminin-411, 2.85 ug/mL; Type-IV collagen, 250 ng/mL. Cells suspended in Opti-MEM-I or Opeguard-MA were bound to laminin, yet no binding was observed in cells suspended in BSS-Plus. Both the fully differentiated, mature cHCEC subpopulations and the epithelial-to-mesenchymal– transitioned (EMT)-phenotype subpopulation were found to attach to laminin- or collagen-coated plates. Interestingly, the binding properties to laminins differed among those subpopulations. Although the level of cells adhered to the laminin-411–coated plate was the same among the cHCEC subpopulations, the fully differentiated, mature cHCEC subpopulations was significantly more tightly bound to laminin-511 than was the EMT-phenotype subpopulations. These findings suggest that the binding ability of cHCECs to major Descemet's membrane components is distinct among cHCEC subpopulations, and that Opti-MEM-I and Opeguard-MA are useful cell-suspension vehicles for cell-injection therapy. This research group focused on developing a novel medical approach, termed cell-injection therapy, for the treatment of patients with endothelial dysfunction.

BioLamina
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Cancer Applications

A laminin 511 matrix is regulated by TAZ and functions as the ligand for the a6Bb1 integrin to sustain breast cancer stem cells
Chang C., Lal Goel H., Gao H., Pursell B., Shultz L.D., Greiner D.L., Ingerpuu S., Patarroyo M., Cao S., Lim E., Mao J., Kulju McKee K., Yurchenco P.D., Mercurio A.M.
Research communication, 2015
One of the first papers that highlighted the importance of ECM proteins in 2D breast cancer stem cell culture. Shows that laminin-511 is a critical niche component for breast cancer stem cells. Breast cancer stem cells produce a laminin-511 matrix that functions as the ligand for the a6Bb1 integrin to promote self-renewal and tumor initiation. The authors observed that TAZ regulates the transcription of the a5 subunit of LN511 and the formation of a LN511 matrix. These data establish a positive feedback loop involving TAZ and LN-511 that contributes to stemness in breast cancer. They see down-regulation of the laminin B2 chain.

Polymerized Laminin-332 Matrix Supports Rapid and Tight Adhesion of Keratinocytes, Suppressing Cell Migration
Kariya Y., Sato H., Katou N., Kariya Y., Miyazaki K.
PLOS ONE, 2012
Laminin-332 is known to supports the stable anchoring of basal keratinocytes to the epidermal basement membrane but is also motility factor for wound healing and cancer invasion. Here they investigated Laminin-332 matrices deposited by normal human keratinocytes and several cancer cell lines. All types of the cells efficiently deposited Laminin-332 on the culture plates in specific patterns. On the contrary, laminins containing laminin ß1 and/or c1 chains (such as LN511 and LN311) were not deposited on the culture plates even if secreted into culture medium. The deposited Laminin-332 matrix showed a mesh-like network structure as analyzed by electron microscopy, suggesting that LN332 was highly polymerized. Laminin-332 matrix rather suppressed the migration of keratinocytes as compared with purified LN332 (not a BioLamina product), which highly promoted the cell migration. The LN332 matrix supported adhesion of keratinocytes much more strongly and stably than purified LN332. Integrin a3ß1 bound to the LN332 matrix at a three times higher level than purified LN332. These results indicate that the polymerized LN332 matrix supports stable cell adhesion whereas unassembled soluble LN332 supports cell migration. The question is though how the purified Ln-332 looked like. Difficult to purify and might be fractionated.

Laminin-332 sustains chemoresistance and quiescence as part of the human hepatic cancer stem cell niche
Govaere O., Wouters J., Petz M., Vandewynckel Y-P., Van den Eynde K., Van den broeck A., Verhulst S., Dollé L., Gremeaux L., Ceulemans A., Nevens F., van Grunsven L.A., Topal B., Vankelecom H., Giannelli G., Van Vlierberghe H. Mikulits W., Komuta M., Roskams T.
Journal of hepatology, 2015
This study demonstrates that tumor behavior is plastic and depends on the microenvironment of the tumor cell. We particularly identified an important role for LN-332 and more specifically its gamma2-chain as part of the specialized cancer stem cell niche in maintaining and supporting 'stemness', e.g. quiescence and chemo-resistance. Ln-332 induces K19 expression, quiescence and chemo-resistance *in vitro.* LN-332 not only protects hepatic cancer cells against chemotherapy but stimulates cell proliferation upon sorafenib exposure. Therefore, monoclonal antibody treatment targeting the gamma2-chain of LN-332 could provide an innovative therapy of hepatic cancer.

Collaboration of 3D Context and Extracellular Matrix in the Development of Glioma Stemness in a 3D Model
Ma N.K.L., Kai Lim J., Fatt Leong M., Sandanaraj E., Ti Ang B., Tang C., Wan A.C.A.
Biomaterials, 2015
The results demonstrate how 3D versus 2D context profoundly affects ECM signalling, leading to stemness. U251 glioblastoma cells were cultured on electrospun polystyrene (ESPS) scaffolds coated with 7 different laminin

isoforms (LAMscreen kit) to provide a 3D model for stem cell-related genes and proteins expression studies. The authors observed collaboration between 3D context and laminins in promoting glioma stemness. The results indicate the influence of 3D (versus 2D) context on stemness markers and integrin expression, specifically, the upregulation of the laminin-binding integrins a6b4. Enhanced clonogenicity of cells grown on ESPS scaffolds in collaboration with laminins 411, 421, 511 and 521.

Integrin-dependent response to laminin-511 regulates breast tumor cell invasion and metastasis
Kusuma N., Denoyer D., Eble J.A., Redvers R.P., Parker B.S., Pelzer R. Anderson R.L., Pouliot N.
International Journal of Cancer, 2011
Laminin-511 is a potent adhesive and migratory substrate for metastatic breast tumor cells in vitro and its expression correlates with tumor grade and metastatic potential in vivo. Here the authors compared the metastatic potential of 4T1 mammary carcinoma cells to that of 4T1 variants isolated by repeated chemotactic migration toward LM-511 in vitro (4T1LMF4) followed by serial injection into the mammary gland and recovery of spontaneous metastases from bone (4T1BM2). Variant subpopulations exhibited a distinct morphology on LM-511 and increased expression of b1 and b4 integrins compared to parental 4T1 cells. Importantly, mice inoculated with 4T1LMF4 and 4T1BM2 variants showed a 2.5- to 4-fold increase in the incidence of spontaneous metastasis to bone compared to 4T1 tumor-bearing mice. Functionally, 4T1BM2 variants were more adherent and more invasive toward LM-511 than parental 4T1 cells. Treatment of 4T1BM2 cells with lebein-1, a disintegrin with selectivity toward LM-type integrin receptors, potently inhibited their migration and invasion toward LM-511. Similarly, a3b1 integrin-dependent migration and invasion of human MDA-MB-231 breast carcinoma cells toward LM-511 were significantly inhibited by lebein-1. Taken together, these results provide strong evidence that LM-511 contributes to the metastasis of breast tumors and suggest that targeting integrin-LM- 511 interactions with lebein-1 or other inhibitors of LM-511 receptors may have therapeutic potential for patients with advanced breast cancer.

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Animal Applications

Laminin-511 but not -332, -111, or -411 enables mouse embryonic stem cell self-renewal in vitro
Domogatskaya A., Rodin S., Boutaud A., Tryggvason K.
Stem Cells, 2008
Different laminin isoforms, LN-511, -332, -411 and -111, and Matrigel, gelatin and poly-D-lysine are compared as substrata maintaining pluripotent mouse ES cells in vitro without addition of leukemia inhibitory factor. Conclusions are that only LN-511 is able to sustain self-renewal for up to 169 days of culturing and cells maintain expression of pluripotency markers and can be used for generation of chimeric mice.

The ability of inner-cell-mass cells to self-renew as embryonic stem cells is acquired following epiblast specification
Boroviak T., Loos R., Bertone P., Smith A. and Nichols J.
Nature Cell Biology, 2014
The authors show that mouse ICM cells from early blastocysts can progress to ERK independence if provided with a specific laminin substrate. We show by RNA sequencing that Laminin-511 is expressed in the early ICM, as is B1-integrin, which mediates laminin binding in ESCs. No expression of Laminin-521 in mouse ICM. Single ESC derivation in 2i-LIF on a fibronectin-LN511 mix and clonal ESC lines expansion on LN-511.

Enhanced reseeding of decellularized rodent lungs with mouse embryonic stem cells
Lecht S., Stabler C.T., Rylander A.L., Chiaverelli R., Schulman E.S., Marcinkiewicz C., Lelkes P.I.
Biomaterials, 2013
All current decellularization methodologies result in significant alterations of the contents, and ratios of ECM proteins, though the exact degree of the loss of ECM glycoproteins, such as laminins. Pre-treatment of decellularized matrices with defined ECM proteins, to evaluate the efficacy of reseeding of mESC. Following ECMs were tested: bovine collagen type IV; human collagen type IV; human pro-collagen type I; human collagen type I; rat collagen type I; human plasma-purified fibronectin; human thrombospondin-1; VCAM; vitronectin; bovine elastin; Matrigel-purified laminin 111; and human recombinant laminins 111, 211, 332, 411, 421, 511, 521. mESCs lack major integrin receptors for collagens but express high levels of functional receptors for LM and FN. **Laminin next to FN appears to be the major pro-adhesive ECM protein for mESCs.** They evaluated a series of recombinant LMs representing the diversity of isoforms present in the lung and evaluated their affinity towards mESCs adhesion. **The mESCs were found to adhere differentially in an isoform-dependent manner with the following order of potencies 511 = 521 >332 >421 >211 >111 >411.** These findings further support the notion that a3b1 and a6b1 integrin receptors expressed on the mES cell surface are involved in the specific binding to LM in general and to LM 511 and 521.

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Laminin Methods

3D CULTURE

Gelatine methacrylamide-based hydrogels – an alternative 3D cancer cell culture system
Kaemmerer E., Melchels F.P.W., Holzapfel B.M, Meckel T., Hutmacher D.W., Loessner D.
Acta Biomaterialia, 2014
The authors present a 3D biomaterial platform for the analysis of ovarian cancer spheroid growth that is an efficient semi-synthetic alternative, combining native ECM components and tuneable matrix properties, resulting in higher reproducibility, less complexity and better comparability between different groups than traditional cell monolayer approaches. In this study, gelatine methacrylamide-based hydrogels (GelMA) with added LN-411 were established as in vitro and in vivo spheroid-based 3D cancer models.

Modifying alginate with early embryonic extracellular matrix, laminin, and hyaluronic acid for adipose tissue engineering
Chen Y-S., Chen Y-Y., Hsueh Y-S., Tai H-C., Lin F-H.
J Biomed Mater Res Part A, 2015
In this study, a new material, HA-L-Alg, was synthesized by linking developmentally essential ECM constituents hyaluronic acid (HA) and laminin (Life technologies) to alginate (Alg). The fabrication of HA-LAlg was confirmed by FTIR spectroscopy, and it was used to form 3D cell-carrying beads. HA-L-Alg beads had a steady rate of degradation and retained 63.25% of mass after 9 weeks. HA-L-Alg beads showed biocompatibility comparable to beads formed by Alg-only with no obvious cytotoxic effect on the embedded 3T3-L1 pre-adipocytes. HA-L-Alg encapsulated 3T3-L1 cells were found to have a higher proliferation rate over those in Alg-only beads. These cells also showed better differentiation capacity after 2 weeks of adipogenic induction within HA-L-Alg beads. These results support that HA-L-Alg facilitated cell survival and proliferation, as well as stimulated and maintained cell differentiation.

COATING GLASS

Live visualization of chromatin dynamics with fluorescent TALEs
Miyanari Y., Ziegler-Birling C., Torres-Padilla M.E.
Nature structural & molecular biology, 2013
The authors of these two Nature publications show that laminin can be coated directly on glass, which many other substrates and proteins cannot. This enables growth of pluripotent stem cells as monolayeres even on glass, which is especially suitable for live imaging.

Control of ground-state pluripotency by allelic regulation of Nanog
Miyanari Y., Torres-Padilla M.E.
Nature Letter, 2012
Laminin-511 used to coat glass-bottomed dishes.

A Balance between Secreted Inhibitors and Edge Sensing Controls Gastruloid Self-Organization
Etoc F., Metzger J., Ruzo A., Kirst C., Yoney A., Ozair Z.M, Brivanlou A.H., Siggia E.D
Developmental cell, 2016
Used colonies of hESC grown on micropatterned substrate and differentiated with BMP4 to model patterning events in the human embryo. Coated micropatterned glass coverslips with LN521.

Inferring Cell-State Transition Dynamics from Lineage Trees and Endpoint Single-Cell Measurements
Hormoz S., Singer Z.S., Linton J.M., Antebi Y.E., Shraiman B.I., Elowitz M.B.
Cell systems, 2016
Here the authors cultures mouse E14 cells on laminin-511 coated 24-well glass bottom plates (MatTek) for time lapse microscopy and single-molecule fluorescence in situ hybridization imaging.

CRYOPREERVATION

Optimization of slow cooling cryopreservation for human pluripotent stem cells
Miyazaki T., Nakatsuji N., and Suemori H.
Genesis, 2013
This is one of the first customer publications that demonstrates Laminin-521 as an optimal xeno- and feeder-free matrix for pluripotent stem cells. The authors show cells should be cryopreserved as single cells for highest survival which is specifically supported by Laminin-521 that promotes adhesion and self-renewal of fully dissociated single cells in the absence of ROCK inhibitor. They demonstrate 80-90% survival of hPSCs post-thawing and 60% survival following subculture on Laminin-521, allowing for efficient and easy handling of cells and bulk storage of high-quality hPSCs.

TRANSWELL & STRETCH CHAMBER COATING

CCR2+CCR5+ T Cells Producing Matrix Metalloproteinase-9 and Osteopontin in the Pathogenesis of Multiple Sclerosis
Sato W., Tomita A., Ichikawa D., Lin Y., Kishida H., Miyake S., Ogawa M., Okamoto T., Murata M., Kuroiwa Y., Aranami T., Yamamura T.
Journal of Immunology, 2012
To recapitulate the glia limitans layered with parenchymal basal lamina experimentally, we coated the upper sides of Transwell membrane inserts. The upper sides of Transwell membrane inserts (8 mm; Corning) were coated with 10 mg/ml laminin-1 (Sigma) or 20 mg/ml laminin-121. After aspirating the laminin solutions, the membrane inserts were turned upside down, and normal human astrocytes (NHA) were seeded on the lower sides of the membrane inserts. T cells were stimulated, harvested, suspended in the fresh medium, and seeded onto the upper chambers. After 8 h, cell suspension was collected from the lower chambers after careful pipetting, and absolute numbers of migrated cells were calculated. The T-cell migration across the NHA layered with laminin-111 or -121 was less efficient compared with the migration across the untreated membrane or the membrane treated with laminin alone, thus, this model would exhibit barrier functions against the penetration of activated T cells.

A Balance between Secreted Inhibitors and Edge Sensing Controls Gastruloid Self-Organization
Etoc F., Metzger J., Ruzo A., Kirst C., Yoney A., Ozair Z.M, Brivanlou A.H., Siggia E.D
Developmental cell, 2016
Used colonies of hESC grown on micropatterned substrate and differentiated with BMP4 to model patterning events in the human embryo. Coated Costar Transwells with LN521.

TC3. TRPC3-GEF-H1 axis mediates pressure overload-induced cardiac fibrosis
Numaga-Tomita T., Kitajima N., Kuroda T., Nishimura A., Miyano K., Yasuda S., Kuwahara K., Sato Y., Ide T., Birnbaumer L., Sumimoto H., Mori Y., Nishida M.
Scientific Reports, 2016
iPSC-derived cardiomyocytes seeded as single cells onto laminin-211 coated stretch chamber dishes (Menicon).

EMBRYOID BODY FORMATION

A High Proliferation Rate is Critical for Reproducible and Standardized Embryoid Body Formation from Laminin-521-Based Human Pluripotent Stem Cell Cultures
Dziedzicka D., Markouli C., Barbé L., Spits C., Sermon K., Geens M.
Stem Cell Rev and Rep., 2016
In this study the authors developed an efficient and standardized embryoid body (EB) formation protocol for human pluripotent stem cells (hPSC) cultured in a laminin-521-based xeno-free system. The cell proliferation rate of the cells growing on laminin-521 strongly affected the efficiency of aggregate formation, and recently passaged cells, as well as the addition of ROCK inhibitor, were essential for reproducible EB formation from hPSC single-cell suspensions. EBs could be obtained in a variety of differentiation media, in 96-well round-bottom plates and in hanging drops. The authors also showed that the medium used for differentiation influenced the differentiation

outcome to a much greater extent than the number of cells used for the initial EB formation.

MICROPATTERNING

Self-organization of human embryonic stem cells on micropatterns
Deglincerti A., Etoc F., Guerra C.M., Martyn I., Metzger J., Ruzo A., Simunovic M., Yoney A., Brivanlou A.H. Siggia E., Warmflash A.
Nature protocol, 2016
Here, the authors developed a reproducible in vitro protocols that allow the study of spatial organization associated with this developmental transition. They use a micropatterning approach in which human embryonic stem cells are confined to disk-shaped, submillimeter colonies. The protocol takes 3 d; it uses commercial microfabricated slides (from CYTOO), human laminin-521 (LN-521) as extracellular matrix coating, and either conditioned or chemically defined medium (mTeSR). The LN521 coating allows for a simpler coating protocol with robust results. This protocol describes a robust platform for quantitative analysis of the mechanisms associated with pattern formation at the onset of gastrulation.

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Laminin Review Articles

Functional Diversity of Laminins
Domogatskaya A., Rodin S., and Tryggvason K.
Annu Rev Cell Dev Biol., 2012
This extensive review provides in-depth information on the molecular complexity of laminins and the current knowledge of their diversity and different functional roles. The review gives the reader an understanding of the importance of laminins for different cell- and tissue types in both normal and pathological functioning in mammals at different stages of development and function.

Human embryonic stem cells
Damdimopoulou P., Rodin S., Stenfelt S., Antonsson L., and Tryggvason K., Hovatta O.
Best Practice & Research Clinical Obstetrics & Gynaecology, 2015
Short review on establishment of hESC lines on LN-521. Authors state that they easily can establish and expand hESC lines in fully chemically defined animal substance free conditions. hESC lines can be derived from single biopsied cells of embryos that need not be destroyed during the process. The genetic stability and differentiation capacity can be studied. These cell lines can today be safely expanded almost without limitations.