Fig. 1

- cell membrane
- anchoring filament
- lamina densa
- lamina fibroreticularis
- type I collagen fibril

By courtesy of Prof. Eijiro Adachi in Kitasato Univ., Japan
**Fig. 1 : Mouse pancreatic epithelial basement membrane**

The superfine meshwork of lamina densa, which is mainly composed of laminin lattice and type IV collagen meshwork combined with nidogen-1/entactin and perlecan of heparan sulfate proteoglycan, underlies along the basal cell membrane of pancreatic epithelial cells and the both are connected with anchoring filaments.

The other side of lamina densa binds to strong fibrils of type I collagen that endow the surrounding cells integrity against the distortion caused by gravity, through a fine elastic meshwork of lamina fibroreticularis mainly composed of type III and V collagen microfibrils.

The continuous gradient (shift, transition) of superfine lamina densa to coarse type I collagen meshworks endows our tissues the strength and elasticity that enable the resistance against the distortions by gravity and exercise.

Basement membrane is not just a scaffold to present adhesion ligands, but also works as an important matrix that is essential for cell activities such as survival, proliferation, differentiation, and maturation.

**Ref:**

By courtesy of Prof. Eijiro Adachi in Kitasato University, Japan.
**Fig. 2**: De novo synthesis of human basement membrane structure of laminin-α5β1γ1 isoform by cell culture

**Upper left**: The recombinant of HEK293 cells, rLN-10 cells, stably expressing human laminin–α5β1γ1 (hLN-511) formed a lamina densa structure (↑) beneath the basal surface by 2-weeks culture, with the aids of US and EU patents technologies No. 7399634, 7906332, 7972852 and 1437147B. De novo anchoring filaments (△) connected the new lamina densa to the basal surface.

**Upper right**: Immunohistochemistry of major basement membrane components such as laminin (LN) and type IV collagen (Col IV) (↑). The integration proceeded continuously beneath the cell layer.

**Lower left**: The bared lamina densa (↑). By the treatment of alkaline detergent the covering rLN-10 cells were mildly removed without impairment on the underneath lamina densa structure.

**Lower right**: The surface of the bared lamina densa. Through small windows in the lamina densa the underlying type I collagen fibrils were observed.

**Ref**:
**Fig. 3**

CK14 (basal cell)

β-tubulin IV (ciliated cell)

CCSP (clara cell)

MUC5AC (goblet cell)

<table>
<thead>
<tr>
<th>Col I gel</th>
<th>mLN-111 sBM</th>
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<tr>
<td><img src="image1" alt="Image of CK14 expression" /></td>
<td><img src="image2" alt="Image of β-tubulin IV expression" /></td>
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<tr>
<td><img src="image3" alt="Image of CCSP expression" /></td>
<td><img src="image4" alt="Image of MUC5AC expression" /></td>
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Fig. 4

mLN -111 coat

mLN -111 sBM

hLN -511 sBM

50 µm 10 µm 50 µm 10 µm

Airlift culture 7 days 14 days
Fig. 3: Reconstruction of an airway epithelial tissue from subcultured basal cells in vitro

Airway basal cells were prepared from rat trachea by pronase E treatment and subcultured for 2 passages on type I collagen coat. The subcultured epithelial cells were identified by anti-cytokeratin 14 (CK14) antibody and then served for the reconstruction of an airway epithelial tissue in vitro. The cells were seeded on a type I collagen gel (Col I gel) or on the synthesized basement membrane substratum of mouse laminin-111 isoform (mLN-111 sBM), lifted to the air-liquid interface after the confluence, and continued to culture for additional 2 weeks.

Once the basal cells were subcultured on Col I coat, the cells could not recover the polarity shown in vivo tissue, so long as cultured on Col I gel. The CK14-positive basal cells were piled up at random (on Col I gel), but arrayed along the base on mLN-111 sBM. The basal cells could also terminally differentiate to β-tubulin IV-positive ciliated cells as well as CCSP-positive Clara and MUC5AC-positive goblet cells. LN-111 sBM served as a matrix to recover the integrity of an airway epithelial tissue in vivo, but Col I gel failed.

Fig. 4: Differentiation to ciliated cells on sBM substrata, but not on LN-111 coat

The subcultured basal cells could differentiate to ciliated cells by air-liquid interface culture both on mLN-111 and on hLN-511 sBM substrata, but failed on mLN-111 coat.

Both sBM substrata served as a matrix, but mLN-111 coat could not.

Ref:
Fig. 5

Neural progenitor

Neuron
Fig. 5: Differentiation of human ES H9 cell-derived neural progenitor to neuron on hLN-511 sBM substratum

**Upper:** Human ES H9 cell-derived neural progenitor cultured on hLN-511 sBM substratum. The progenitor could be routinely subcultured on the substratum without loss of the potential.

**Lower:** Differentiation of the progenitor to neuron by the addition of BDNF. Even after the differentiation the neuron continued to stably adhere on the substratum. However, the neuron shifted to incidentally detach, once the differentiation was promoted on the poly-L-lysine, mLN-111, and fibronectin coat.

hLN-511 sBM served as a matrix for neural progenitor to proliferate and differentiate to neuron.
Fig. 6
Fig. 6: Differentiation of mouse ES cells to mature hepatocytes on hLN-511 sBM substratum

Upper left: Mouse ES cells were cultured on hLN-511 sBM substratum (Fig. 2) and step by step differentiated to definitive endoderm, hepatocyte progenitor, and finally mature hepatocytes in 30-days culture, with no need of feeder cells instead just by changing the culture medium.

Immunohistochemistry of $\alpha$-fetoprotein (AFP) and albumin (ALB).

Upper center: On day 18 mES cells differentiated to hepatocyte progenitors still with a high expression of AFP and with a lower ALB.

Upper right: On day 30 mature hepatocytes differentiated from mES cells expressed a high level of ALB and a trace of AFP. ▲, binuclear hepatocyte-like cells.

Lower left: The temporal shift of AFP and ALB gene expression along the differentiation of mES cells to mature hepatocytes. FL: fetal liver.

Lower right: The enhancement of ALB secretion activity along the progress of differentiation to mature hepatocytes. FL: fetal liver

hLN-511 sBM worked as a matrix to guide mES cells to the terminal differentiation to hepatocytes without coculture of feeder cells.

Ref: