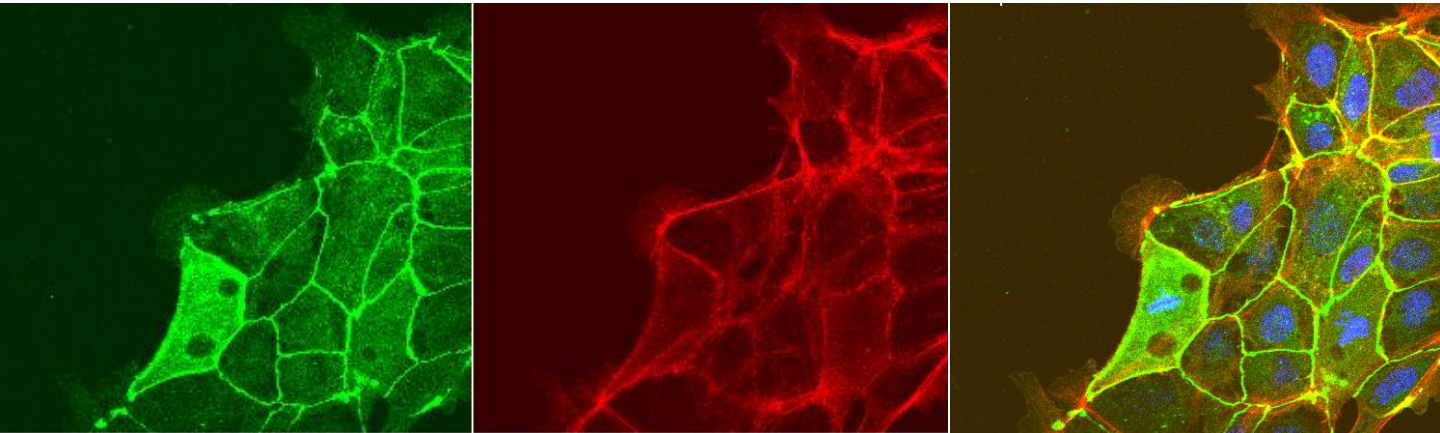


amsbio

Substrate for ES/iPS Cells

iMatrix-511

Recombinant Human Laminin-511 E8 Fragments



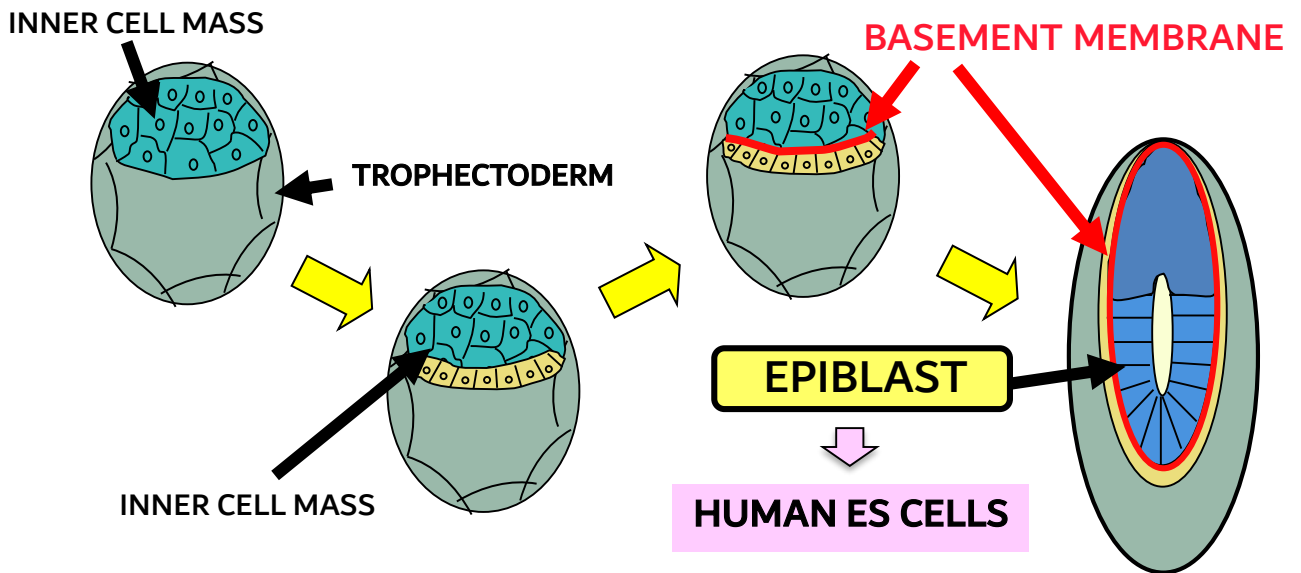
Laminin-5 | | Expressed in Early Development

Day 3.5

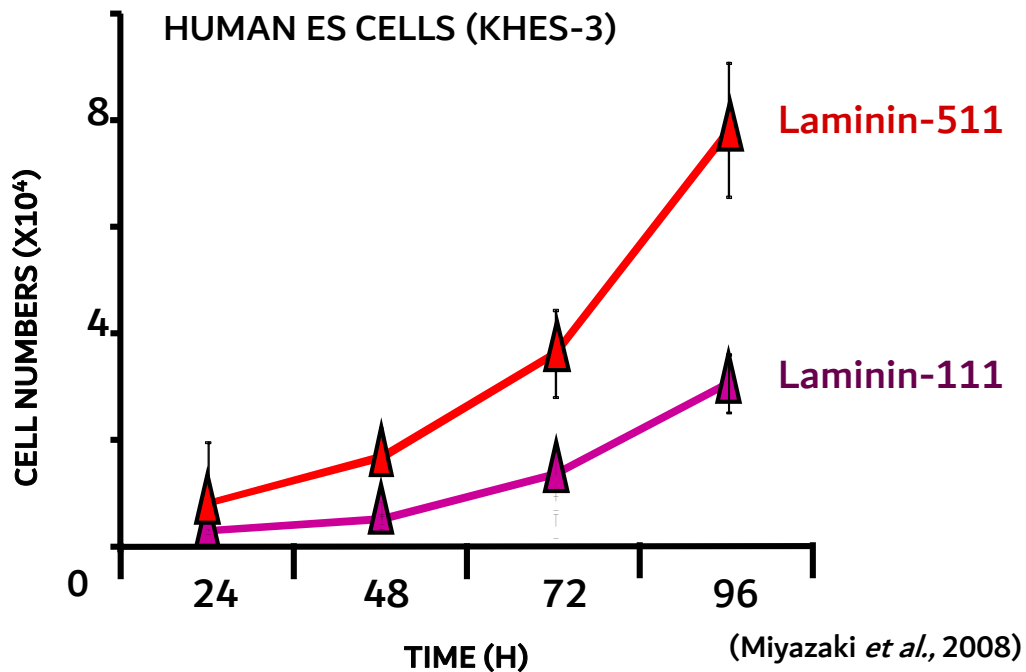
Day 4

Day 4.5

Day 5.5

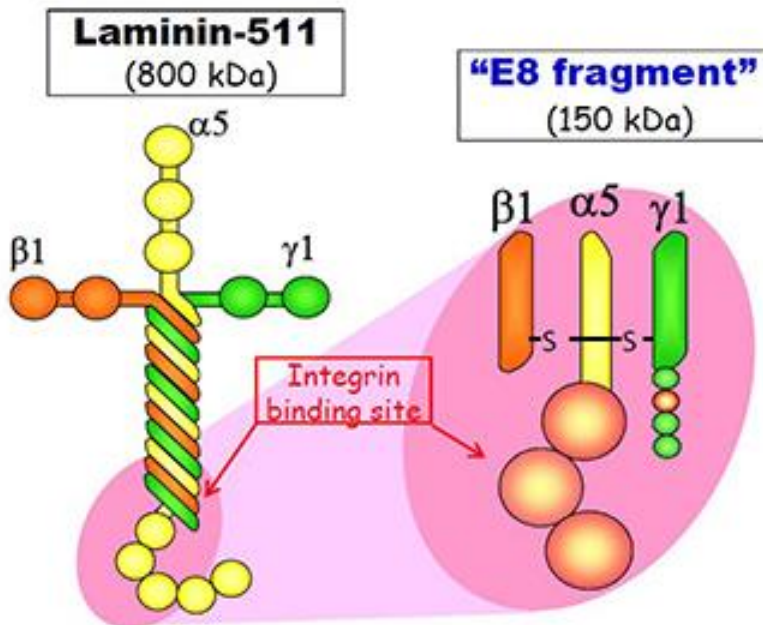


Effect of Laminin-511 on Feeder-Free Stem Cell Culture



Laminin-511 coating enhances the ES cell growth.

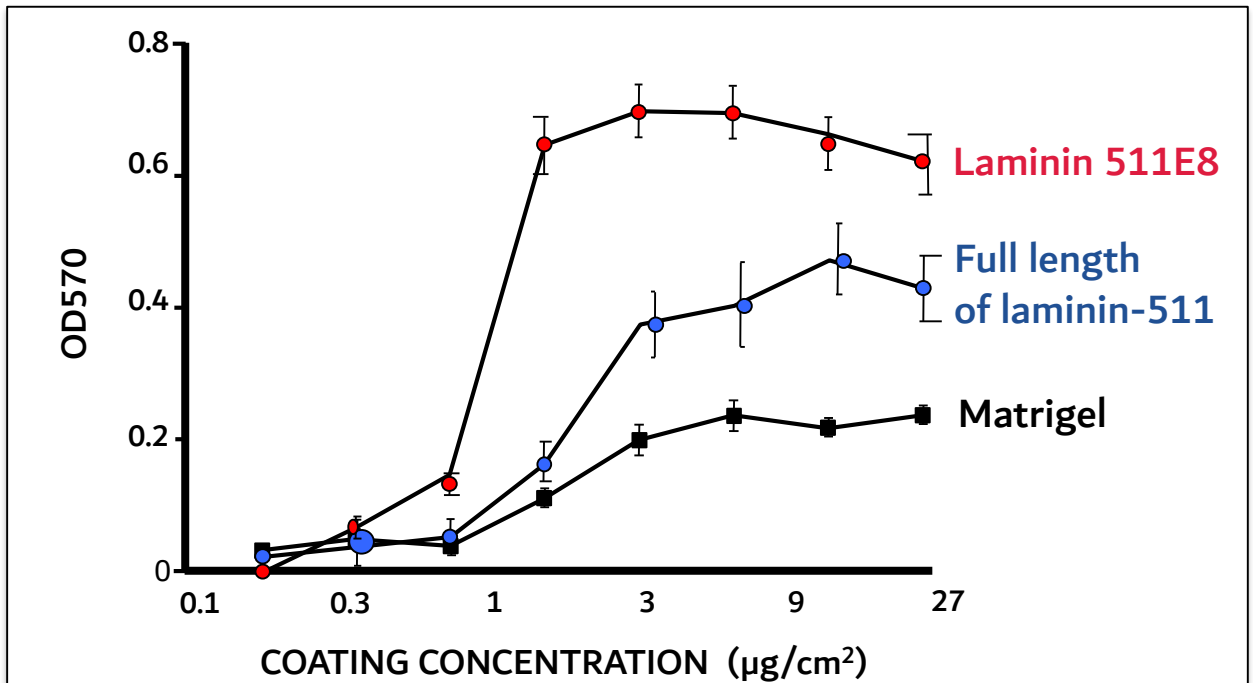
Laminin – More Than 12 Isoforms



| | α | β | γ |
|--------------------|------------------------------|-----------------------------|------------------------------|
| Laminin-111 | $\alpha 1$ | $\beta 1$ | $\gamma 1$ |
| Laminin-211 | $\alpha 2$ | $\beta 1$ | $\gamma 1$ |
| Laminin-121 | $\alpha 1$ | $\beta 2$ | $\gamma 1$ |
| Laminin-221 | $\alpha 2$ | $\beta 2$ | $\gamma 1$ |
| Laminin-332 | $\alpha 3$ | $\beta 3$ | $\gamma 2$ |
| Laminin-311 | $\alpha 3$ | $\beta 1$ | $\gamma 1$ |
| Laminin-321 | $\alpha 3$ | $\beta 2$ | $\gamma 1$ |
| Laminin-411 | $\alpha 4$ | $\beta 1$ | $\gamma 1$ |
| Laminin-421 | $\alpha 4$ | $\beta 2$ | $\gamma 1$ |
| Laminin-511 | $\alpha 5$ | $\beta 1$ | $\gamma 1$ |
| Laminin-521 | $\alpha 5$ | $\beta 2$ | $\gamma 1$ |
| Laminin-213 | $\alpha 2$ | $\beta 1$ | $\gamma 3$ |

E8 Fragment contains the integrin binding site.

Laminin 511 E8 Fragment - the Best Substrate



The binding activity of Laminin 511 E8 Fragment against ES cell was better than Full length 511 and traditional substrate.

The same function was confirmed in human iPS cells

Advantages of iMatrix-511

Efficient bulk proliferation

Ideal for feeder-free cell culture

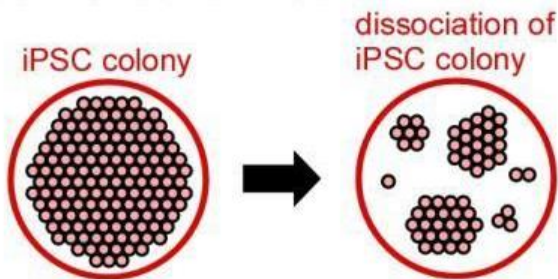
Pre-mix method

Superior adhesion

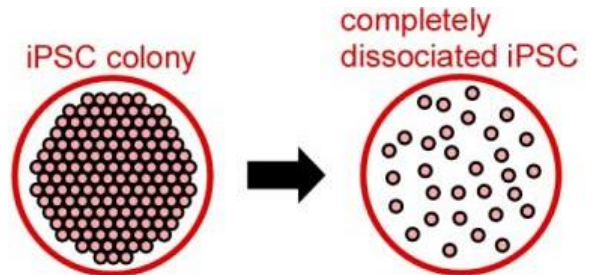
Efficient Bulk Proliferation

- Complete dissociation of iPSC colony results in extensive cell death
- Large aggregates causes spontaneous differentiation

Conventional Method



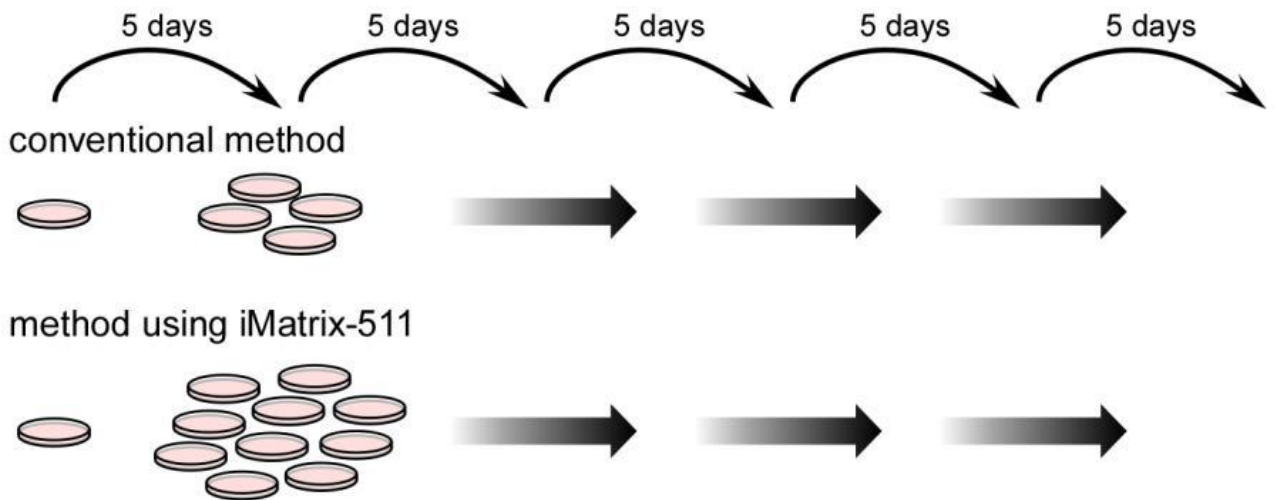
Method using iMatrix-511



Complete dissociation of iPSC colony avoids cell death

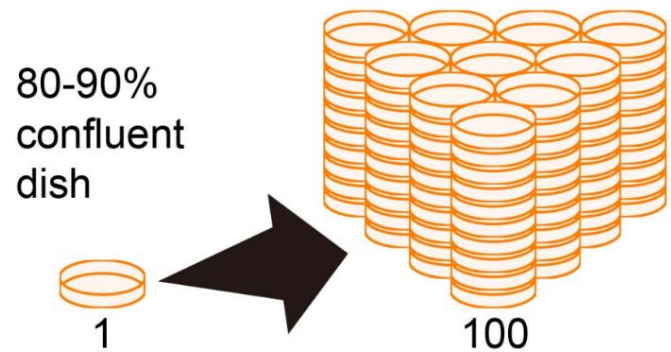
Efficient Bulk Proliferation

iMatrix-511 allowed a higher passaging ratio during subculture, which was approximately **1:100** compared with **1:4** for conventional method.



Split Ratio 1:100 with iMatrix-511

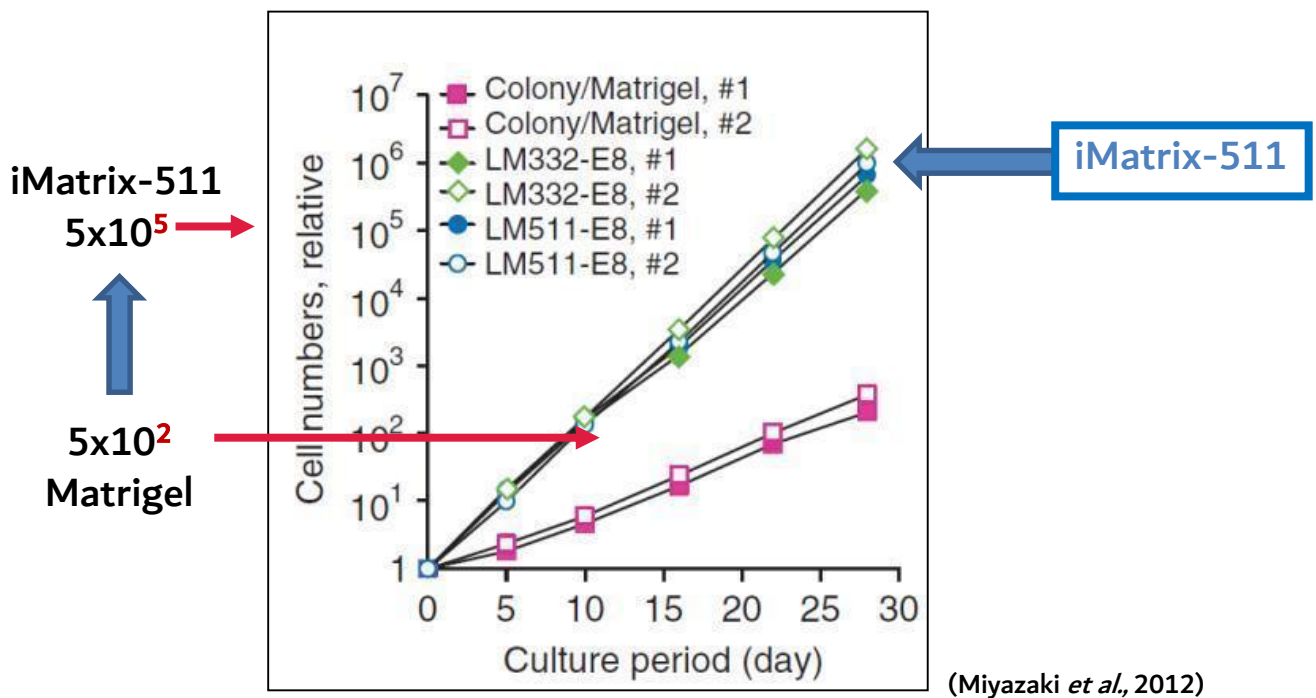
| hESCs or hiPSCs | Doubling Time hrs | Fold Changes /passage |
|-----------------|-------------------|-----------------------|
| KhES1 | 28.34 | 131.23 |
| 1027B6 | 29.05 | 95.85 |
| 1027B3 | 29.37 | 112.31 |
| 987A3 | 26.00 | 156.73 |
| 987A7 | 28.09 | 133.50 |
| 1020A12 | 30.30 | 106.75 |
| 201B7 | 26.90 | 177.49 |
| 201B6 | 28.97 | 124.05 |
| Average | 28.34 | 132.00 |



- The hESCs and hiPSCs were efficiently passaged under the feeder-free system. We calculated the doubling times of the hESCs and hiPSCs and the fold change in the cell number in each passage.
- One confluent dish can be passaged into approximately 100 dishes. (Nakagawa *et al.*, 2014, *Scientific Reports* 4, Article number: 3594)

Efficient Bulk Proliferation

iMatrix-511 allowed a higher passaging ratio during subculture.



1,000-fold after one month culture

Safety and Quality Substrate

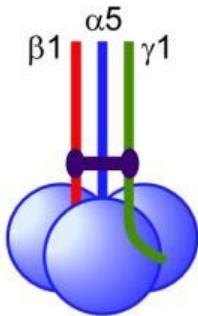


Feeder cells

(fibroblast cell derived from mouse)

Geltrex, Matrigel

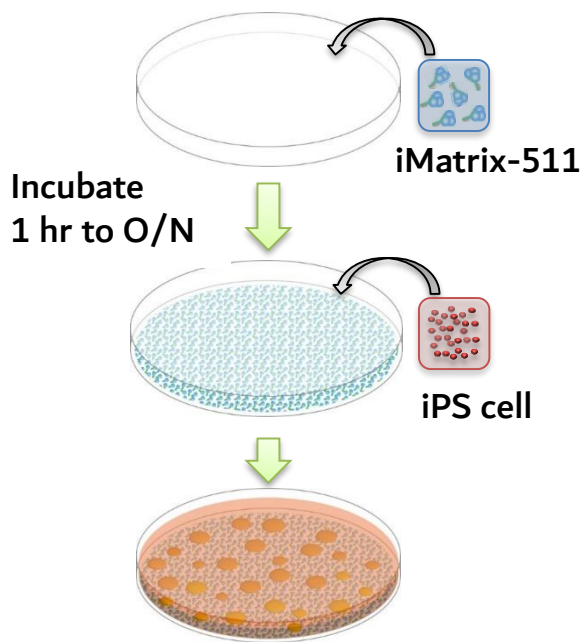
(substrate derived from EHS mouse sarcoma cells)



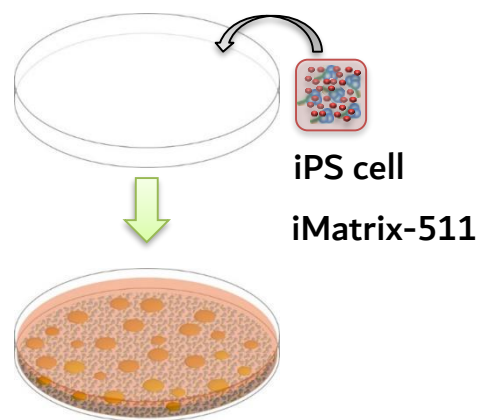
Recombinant human Laminin 511-E8
fragment is made by CHO cell.

Pre-Mix Method

PRE-COAT METHOD



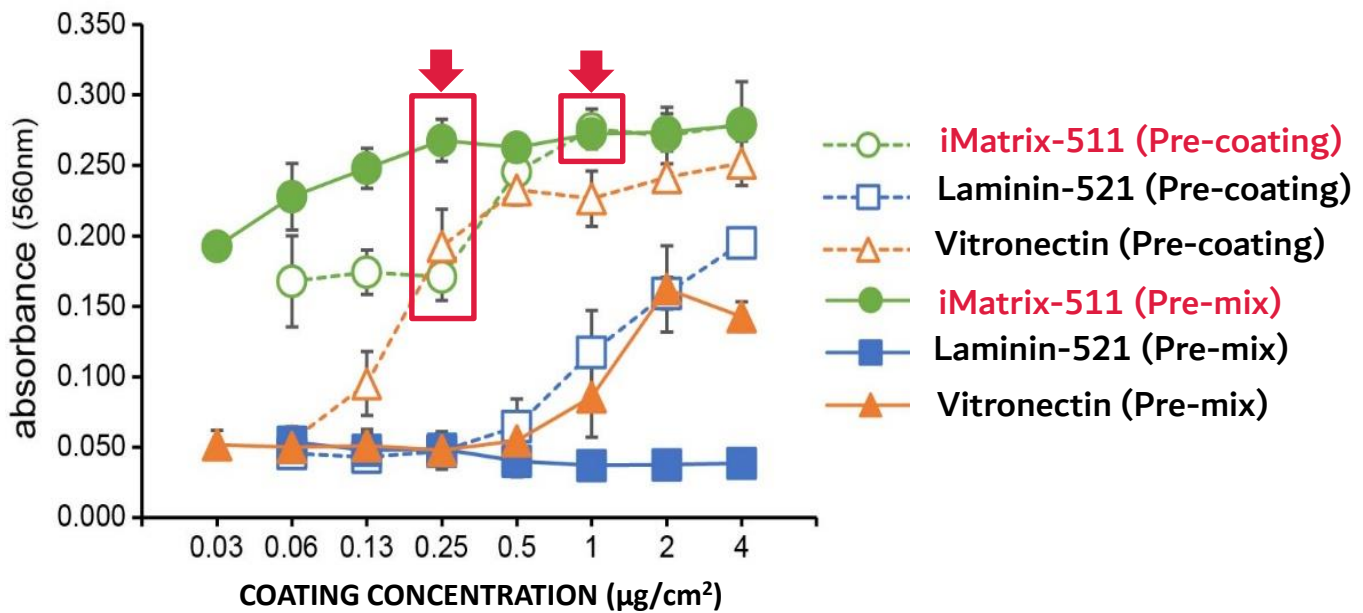
PRE-MIX METHOD



(Miyazaki et al., 2017)

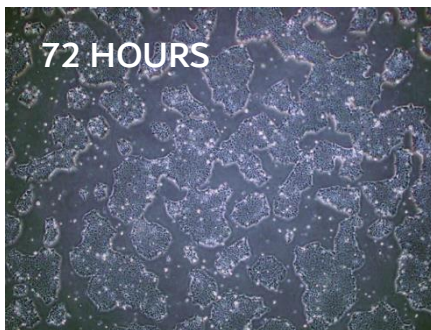
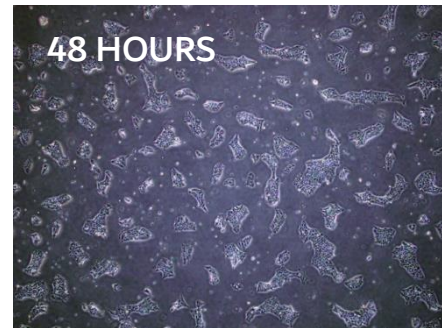
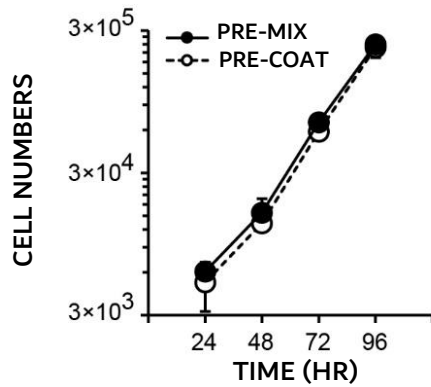
Pre-Mix Method

iMatrix-511 shows better adhesion activity using mix method, rather than the conventional pre-coating method.



By mixing with iMatrix-511, PSC can be immediately pipetted into the fresh dishes.

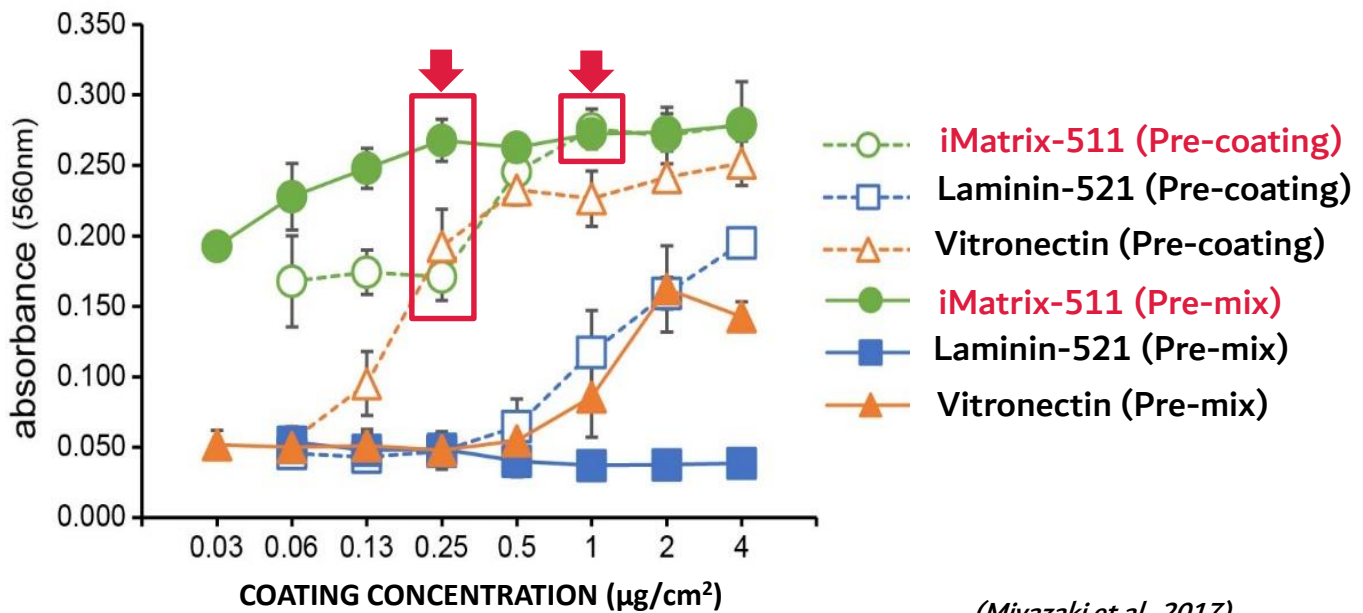
iPS Cell Proliferation Efficiency and Cell Condition



(Miyazaki et al., 2017)

Pre-Mix Method

iMatrix-511 shows better adhesion activity using mix method, rather than the conventional pre-coating method.



(Miyazaki et al., 2017)

By mixing with iMatrix-511, PSC can be immediately pipetted into the fresh dishes.

Advantages in EP/iPS Cell Culturing

No need to pre-coat

- Time saving
- Doesn't waste the coated dishes

Cost efficiency

- Necessary amount used, is a half compared to pre-coat method

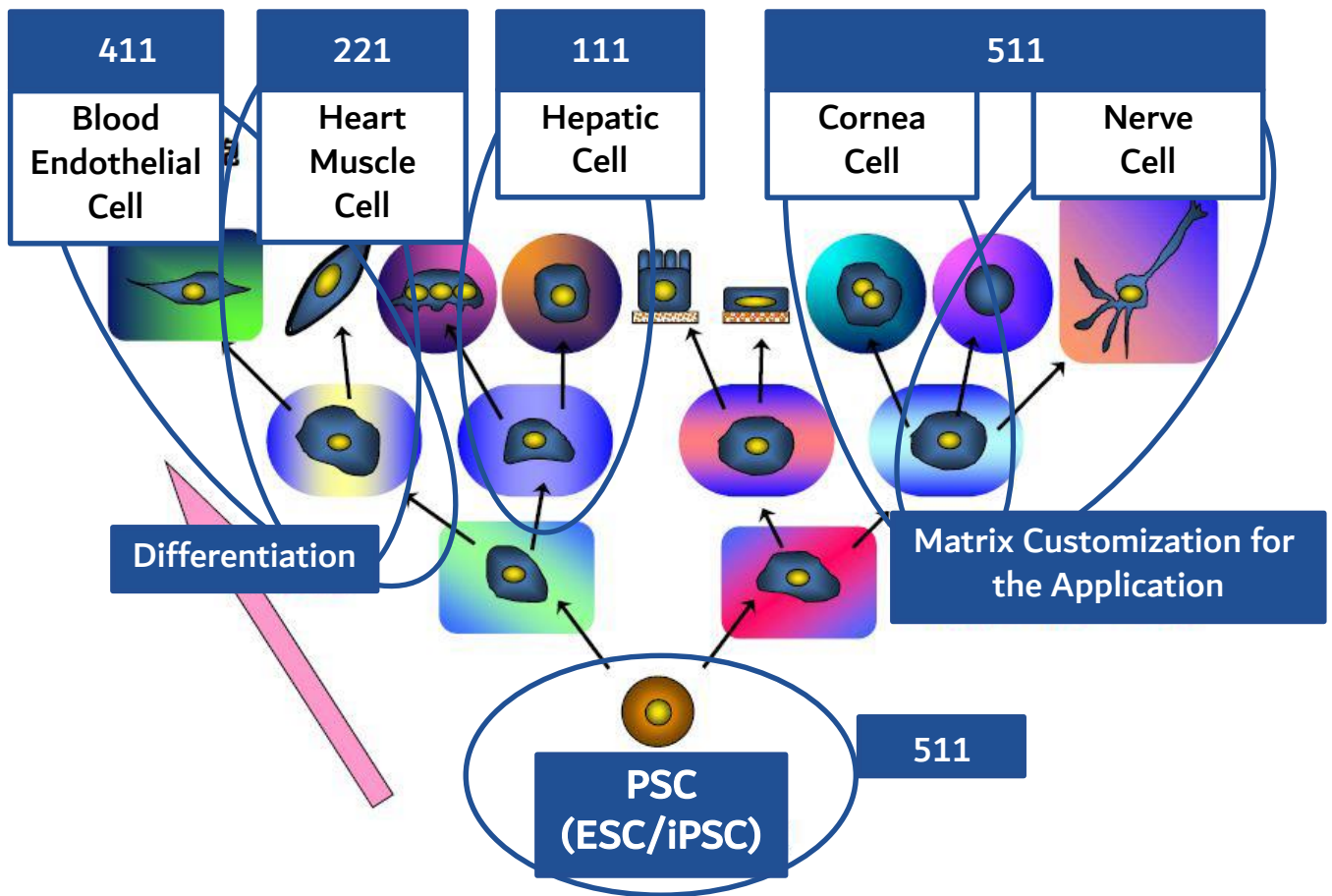
Single cell passaging

- No requirement for colony passaging by skilled technicians

Feeder-Free

- Easy for application in medical trials

Future of Laminin Isoforms Applications



Publication List

- Ido H et al. *J. Biol. Chem.* **282** (15): 11144-54, 2007
- Taniguchi Y et al. *J. Biol. Chem.* **284** (12): 7820-31, 2009
- Miyazaki T et al. *Nat. Commun.* **3**: 1236, 2012
- Nakagawa M et al. *Sci Rep.* **4**: 3594, 2014
- Doi D et al. *Stem Cell Reports.* **2** (3): 337-50, 2014
- Takashima Y et al. *Cell.* **158** (6): 1254-69, 2014
- Fukuta M et al. *PLoS One.* **9** (12) : e112291, 2014
- Burridge PW et al. *Nat. Methods.* **11**: 855-60, 2014
- Okumura N et al. *Invest Ophthalmol Vis Sci.* **56** (5), 2933-42, 2015
- Sasaki K et al. *Cell Stem Cell.* **17**(2), 178-94, 2015
- Hayashi R et al. *Nature* **531**, 367-80, 2016
- Takayama K et al. *Biochem. Biophys. Res. Com.* **474** (1): 91-96, 2016
- Matsuno K et al. *Defferentiation.* 2016
- Samata B et al. *Nat. Commun.* **7**: 13097, 2016
- Miyazaki T et al. *Scientific Reports*, **7**, 41165, 2017
- Goparaju S et al. *Scientific Reports*, **4**, 42367, 2017
- Camp J. G. et al. *Nature*, 2017



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