In vivo Live Imaging of Human iPSC-Derived Endothelial Cells Seeded on Decellularized Small-Diameter Vascular Grafts

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Background

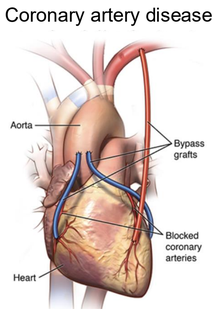
- **Small-diameter vascular grafts are prone to occlusion.**

✓ Despite the frequent clinical need for small-diameter vascular grafts, they are prone to occlusion. To address this longstanding challenge, attempts have been made to pre-seed cells onto the grafts prior to implantation.

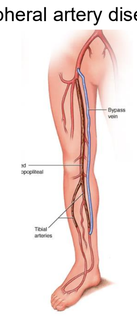
✓ A meta-analysis of 68 preclinical studies demonstrated a significant difference in patency rates between cell-seeded and unseeded grafts (85.1% vs. 62.4%, $p < 0.001$), indicating a clear benefit of cell seeding.

✓ Regarding cell type, endothelial cells were shown to contribute more to improved patency rates than smooth muscle cells.

Coronary artery disease



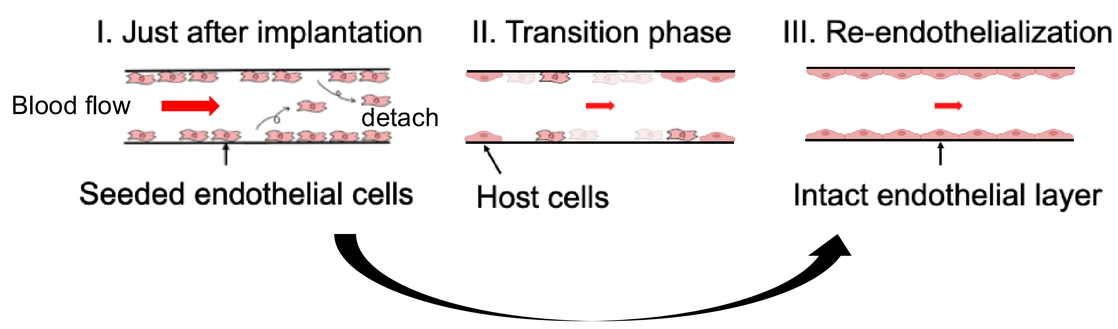
Peripheral artery disease



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Objectives of the Present Study

- **(Known) Seeded cells eventually detach and are replaced by host cells.**



However, the chronological transition from seeded cell detachment to autologous endothelial cell engraftment remains unknown.

Experimental Design

※ EC : Endothelial Cell

Venus-Akaluc-labeled hiPSC-ECs



Decellularized graft

Cell seeding
&
Pre-culture with Bioreactor

implantation



Akalumine



AkaBLI system



Bioluminescence imaging (BLI)

Result 1 : Preparation of Decellularized Grafts

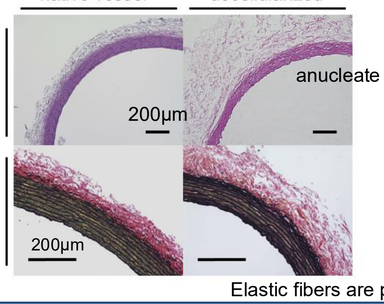
0.5% SDS : 10ml/min



Decellularized vessel



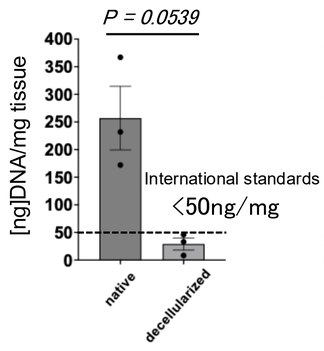
<Histological analysis>



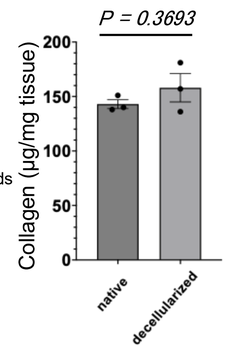
EVG :
Elastica van Gieson stain

Elastic fibers are preserved.

<Residual DNA >

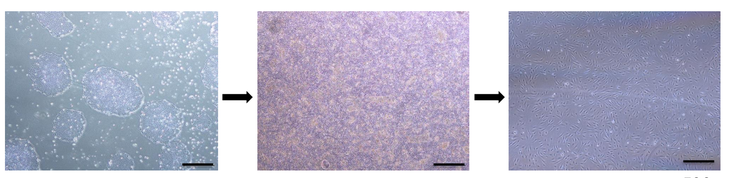


<Residual Collagen>



Result 2 : hiPSC-ECs Differentiation and Reporter Gene Transduction

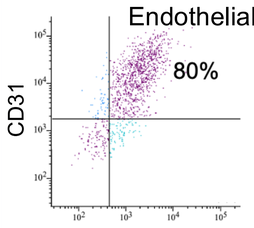
Day(-4) Day(1) Day(14)



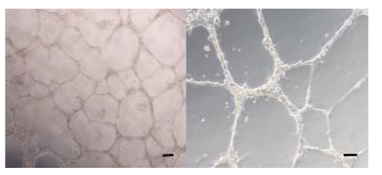
iPS cells (201B7) Endothelial cells

<Flow cytometry>

Endothelial cells

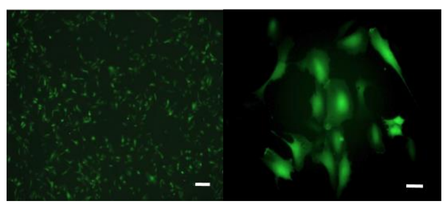


<Tube formation assay>

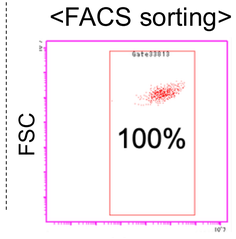


→ functional endothelium

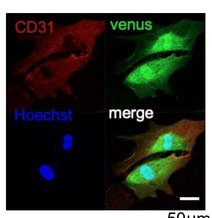
<Venus-Akaluc retroviral transduction>



<FACS sorting>



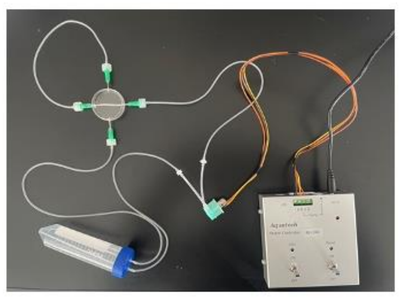
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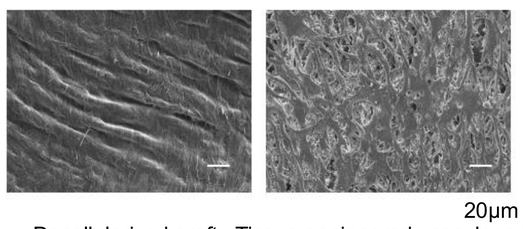
Result 3 : Luminal Seeding and Perfusion Culture of hiPS-ECs

Static culture 2 days
(Cell seeding : 2×10^5 cells/cm^2)
→ Perfusion culture 3 days

Perfusion bioreactor system



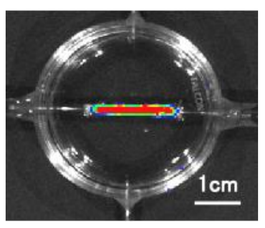
Scanning Electron Microscopy (SEM)



Decellularized graft (DG)

Tissue engineered vascular graft (TEVG)

In vitro evaluation



The luminescent cells have engrafted successfully.

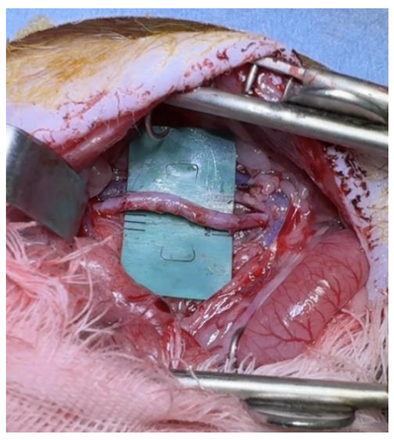
Result 4 : Abdominal Aortic Implantation of the DG / TEVG

Abdominal aorta

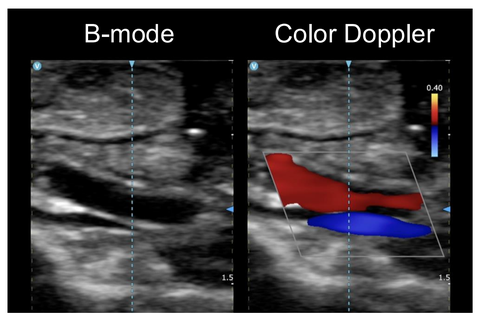


- ✓ Nude rat, F344 (rnu/rnu)
- ✓ ≧12 weeks, ♂
- ✓ Implantation
  →DG vs TEVG group
- ✓ explant : 1 month

Graft implantation



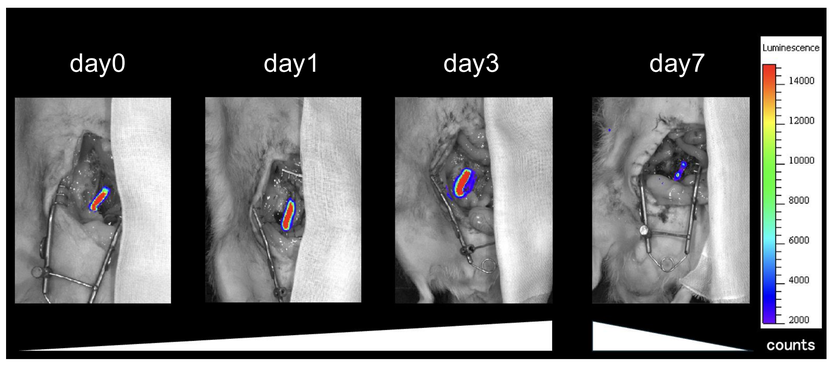
Ultrasonography



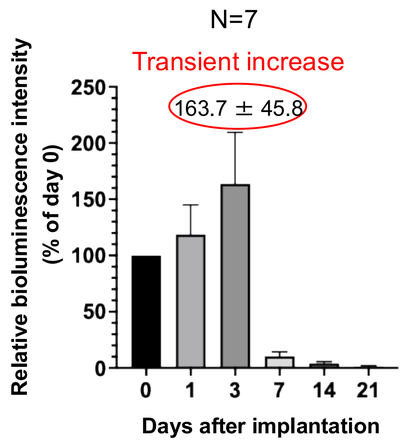
Patency was assessed by ultrasonography.

Result 5 : *In Vivo* Live Imaging of hiPS-EC

<Bioluminescence intensity>



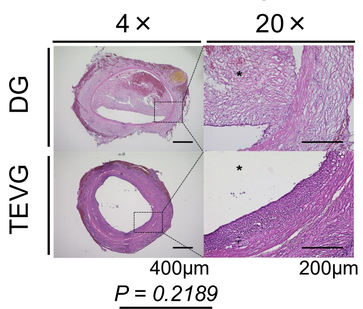
Transient increase↑ Rapid attenuation↓

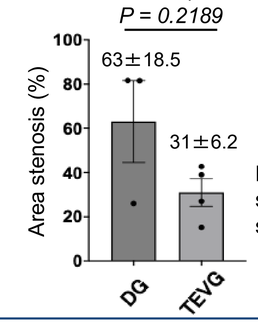


Data : Mean ± SEM

Result 6 : Histological Analysis at 1 Month After Implantation

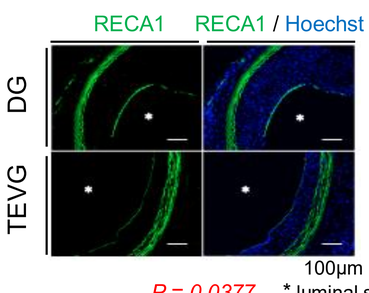
HE staining

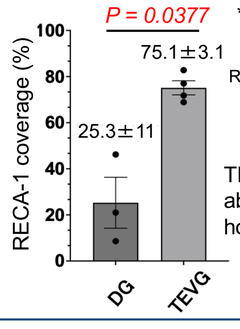




DGs tended to show progressive stenosis.

IF : Endothelial cell marker

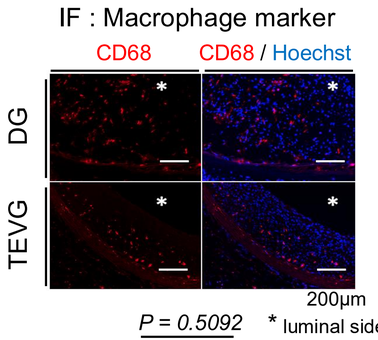


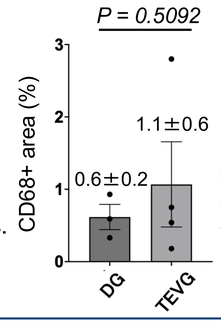


RECA1 : Rat Endthelial Cell Antigen1

TEVGs have the ability to recruit host endothelial cells.

IF : Macrophage marker





Macrophage marker
→ n.s.

Conclusion

- ➢ BLI revealed a transient peak in luminescence intensity at day 3.
- ➢ Visualizing the duration of engraftment of the seeded cells enables the optimization of currently unestablished cell seeding protocols.
- ➢ hiPSC-ECs promote early endothelialization without excessive inflammation, providing key data for future clinical applications.

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