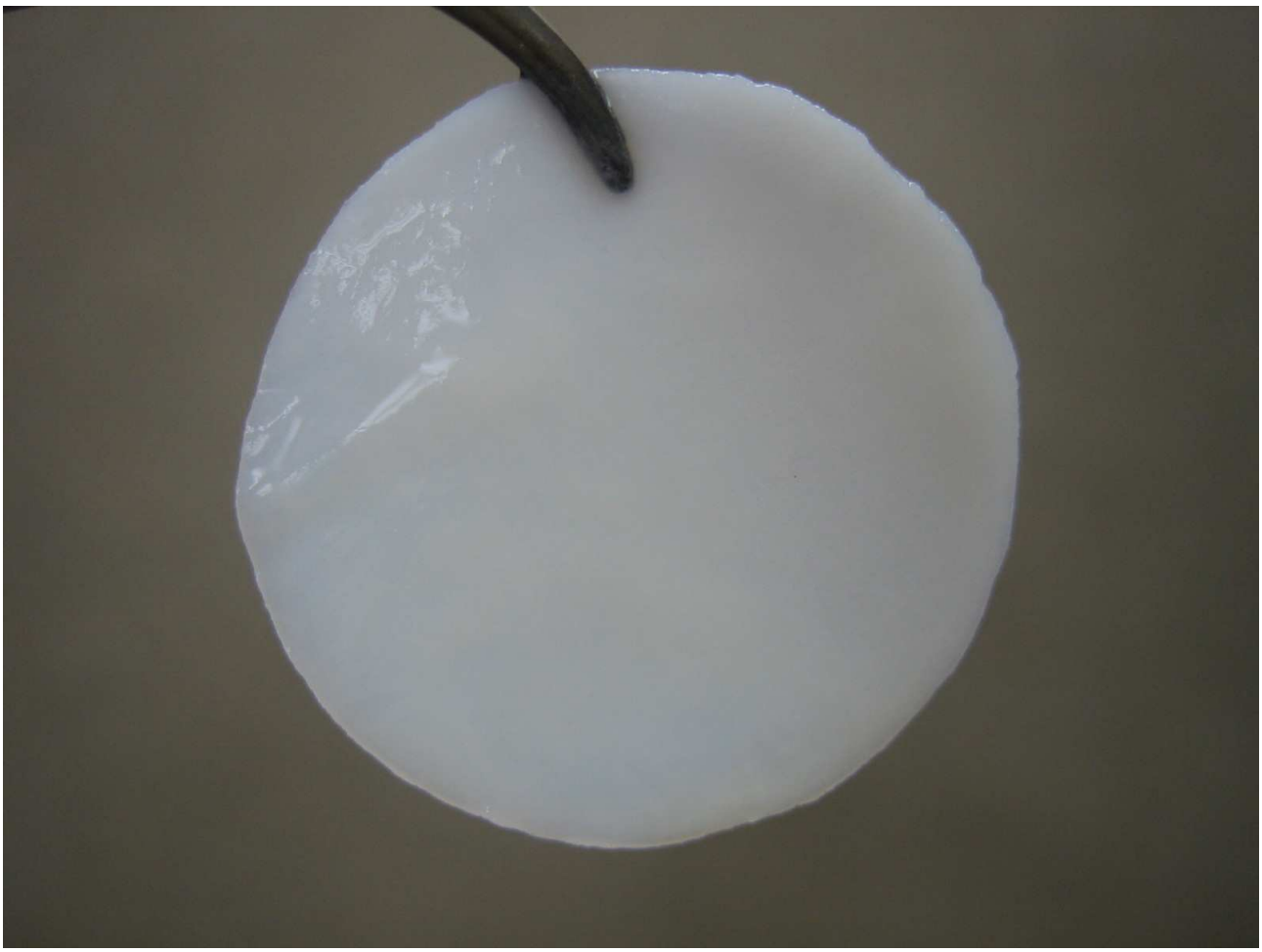
SCETI

Scaffold for cell culture

CardioDISC™



Similar to physiological environment

High cell-affinity

High biocompatibility

Easy to handle

1. Introduction

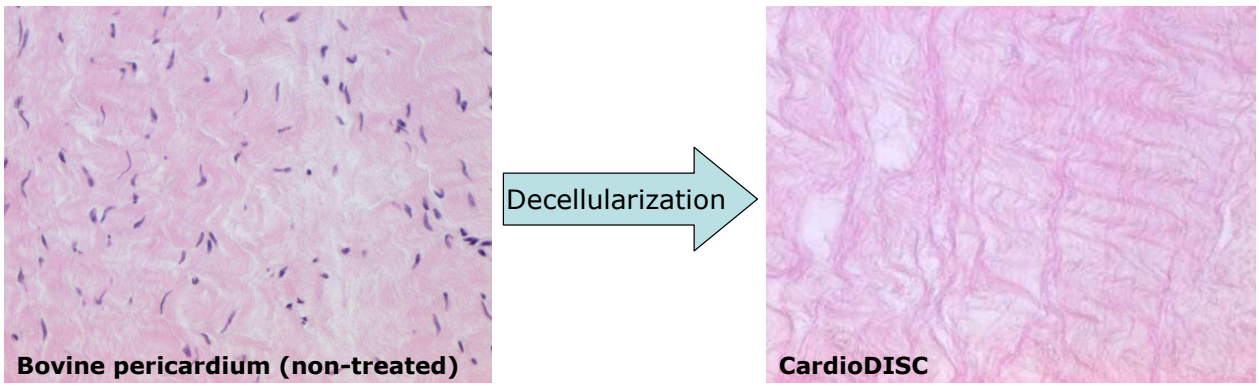
Each organ and tissue function in coordination with undifferentiated and mature cells, extracellular matrix (ECM) and growth factor. Especially, ECM is crucial for the differentiation and function of surrounding cells. ECM molecules influence the intracellular signals, which regulate proliferation, differentiation, migration, and function, through ECM receptors on the cell surface.

To study the physiological function of adherent cells, various ECM substitutes (e.g. ECM coats and 3D-scaffolds) consisting essentially of biological or chemical compounds have been used. However, it is reported that these substitutes are quite different from physiological environment. Moreover, low biocompatibility and difficulty of handling prevent researchers using these substitutes as a scaffold of cell transplantation.

CardioDISC^{TM} is a sheet-formed scaffold for cell culture derived from Japanese bovine pericardium. Our decellularization method leads to the elimination of bovine antigen, the maintenance of native ECM structure and non-residual cytotoxicity. Therefore, CardioDISC^{TM} achieves high cell-affinity and high biocompatibility.

CardioDISC^{TM} supports cell culture experiments using various cell types. CardioDISC^{TM} is also able to be applied to cell transplantation experiments.

Advantages	Features
Non-denaturing ECM	→ Similar to physiological environment
Non-residual cytotoxicity	→ High cell-affinity
Non-residual bovine antigen	→ High biocompatibility
Derived from pericardium	→ Easy to handle



Hematoxylin-eosin staining. Cell nuclei are visualized in blue purple.

2. Features

High cell-affinity

CardioDISC™ consists of non-denatured ECM (Fig. 1). Cells adhere to and proliferate on CardioDISC™ (Fig. 2). Moreover, CardioDISC™ has no cytotoxicity and activity changing the properties of cells (Fig. 3).

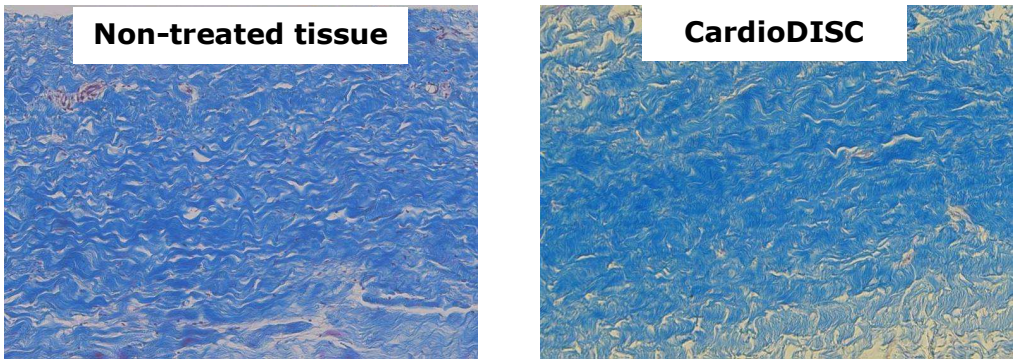


Fig. 1, Collagen in non-treated tissues and CardioDISC™ was visualized by Masson-trichrome staining, showing that CardioDISC™ had the collagen structure as same as non-treated tissues.

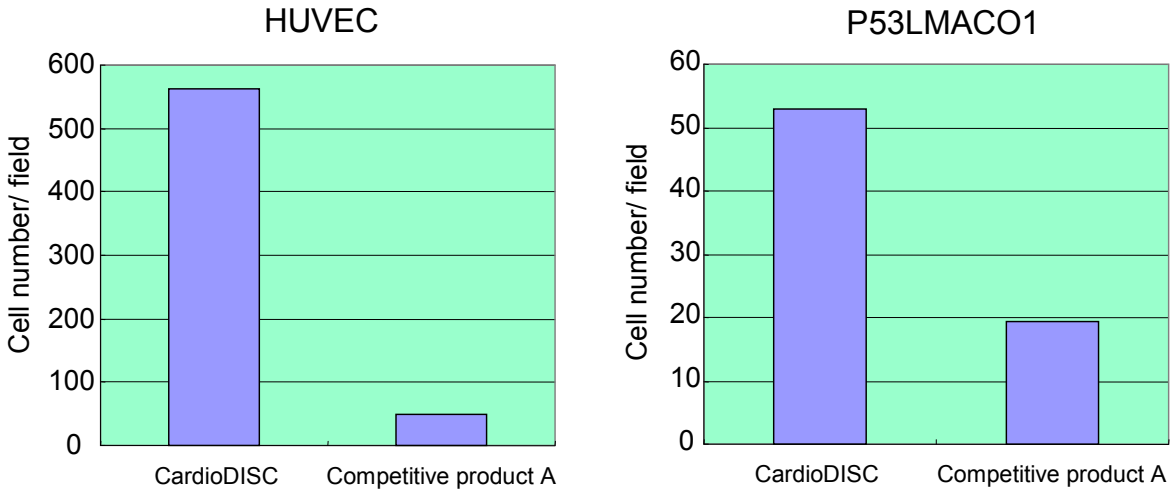


Fig. 2, HUVEC (human umbilical vein endothelial cell) and P53LMACO1 (mouse aorta smooth muscle cell) were cultivated on CardioDISC™ for 14 days, and then the cell numbers of those were counted. Good adhesion and proliferation were observed in both cells.

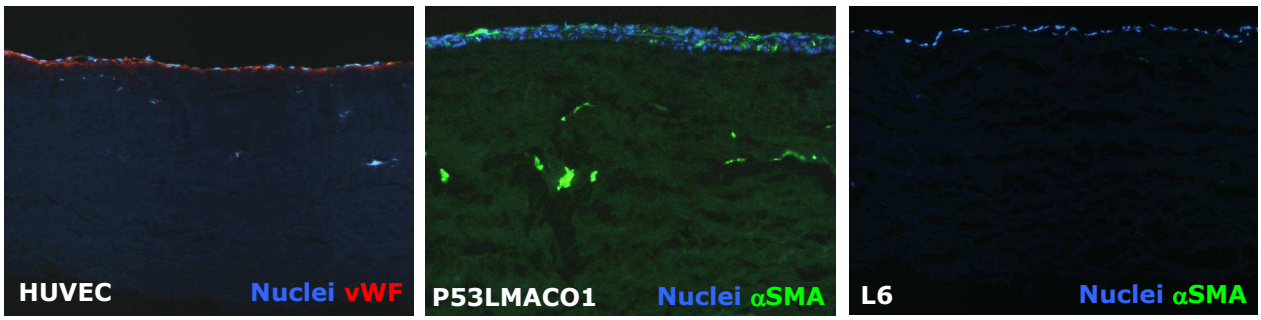


Fig. 3, After cultivating HUVEC, P53LMACO1, and L6 (rat myoblast) on CardioDISC™ for a certain period, the characters of cells were examined by immunofluorescence using each specific antibody. The properties were unchanged before and after the cultivation on CardioDISC™.

High biocompatibility

Antigen in CardioDISC™ was dramatically reduced by the decellularization process (Fig. 4). CardioDISC™ was not rejected and quickly adhere to the surrounding tissues after the implantation (Fig. 5).

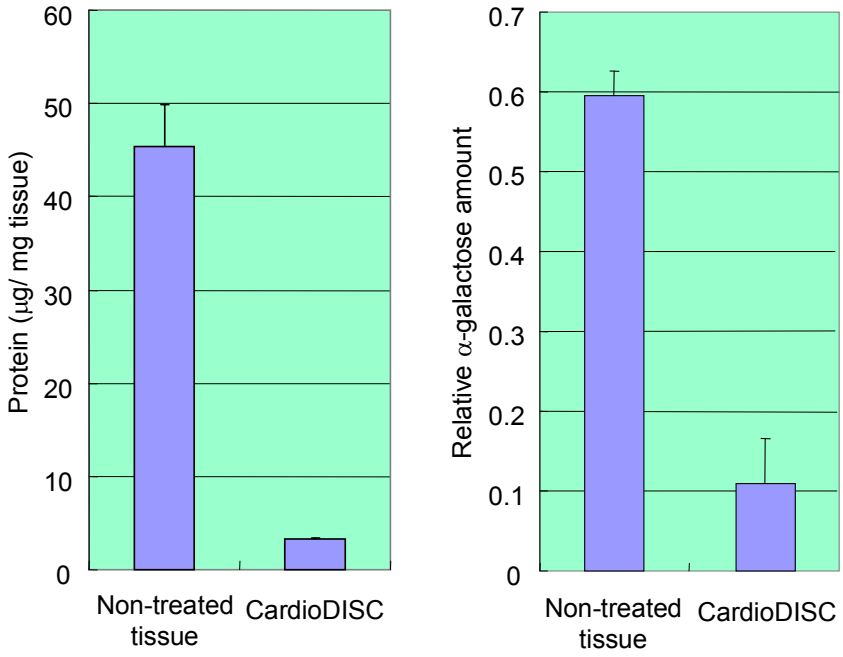


Fig. 4, Amounts of protein and α-galactose (candidates of xenoantigen) in non-treated tissues and CardioDISC™ were measured. Those in CardioDISC™ were drastically decreased compared to the level of non-treated tissues.

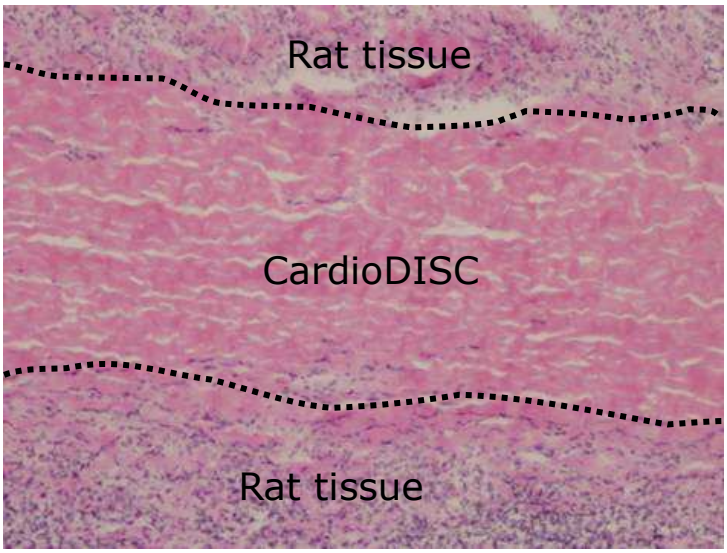


Fig. 5, CardioDISC™ were excised after 2 weeks of subcutaneous implantation in rat back, and observed by hematoxilin-eosin staining. Infiltration of inflammatory cells into CardioDISC™ was hardly observed, and the adhesion of surrounding tissues was in good states.

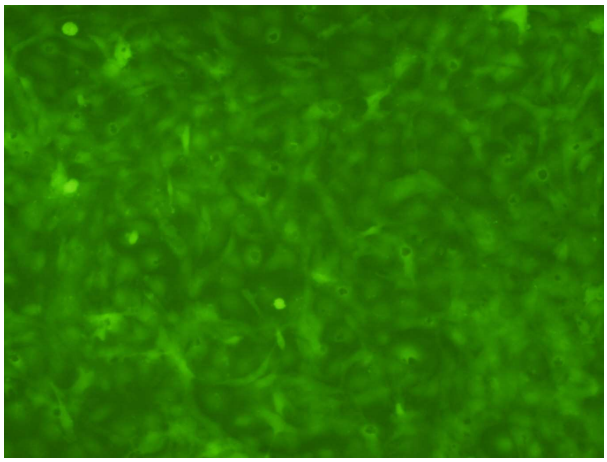
3. Protocol for cultivating vascular endothelial cell using CardioDISC™

Additional materials required

- Pipettes
- 12-well culture plates
- Cell culture medium（EGM-2 supplemented with SingleQuots: manufactured by Cambrex）
- Human umbilical vascular endothelial cells (HUVEC：manufactured by Cambrex）
- CO^2 incubator

Procedure

1. Put CardioDISC™ upper side up on a 12 well culture plate.
2. Add 2 mL warm culture medium, and incubate for 30 min in CO^2 incubator at 37 °C.
3. Discard the culture medium.
4. Seed HUVEC on CardioDISC. A seeding cell number is the option, but 1 x 10^5 cells/well is appropriate.
5. Stir gently a culture plate to disperse the cells uniformly on CardioDISC™. Confirm CardioDISC™ not to float, lean, and bend.
6. On the next day, change to a fresh medium. Culture medium should be changed daily. Cells will be confluent about a week after seeding depending on cell viability. It is ready to use for assay of endothelial cells and implantation into body.



Fluorescent-labeled HUVEC on CardioDISC™ (At 2 weeks after seeding).
Cell layer stably adhered was observed.

Cells confirmed adhering and proliferating on CardioDISC™（Other cells should be confirmed）

HUVEC (human vascular endothelial cells
P53LMACO1 (mouse aorta smooth muscle cells)
NIH3T3 (mouse fibroblast)
L6 (rat myoblast)
293T (Human embryonic kidney cells)

4. Specifications

Product name
CardioDISC™
Product code
C-01-01-EX
Contents
CardioDISC™ φ21mm x 12sheets, Attached paper, Certificate of analysis
Uses
Scaffold for cell culture, organ model, and cell transplantation
Specifications

Origin	: Japanese BSE-free bovine-derived pericardium
Main component	: Collagen
Appearance	: Opal, round-shaped, rubber-like sheet
Diameter	: About 21mm
Thickness	: About 0.4mm
Storage solution	: Phosphate-buffered saline
Wet/dry weight	: About 200mg/50mg

Recommended use temperature
37ºC
Storage temperature
4ºC
Term of validity
6 months (4ºC : Sterility and viability of vascular endothelial cells)
Caution

1. CardioDISC™ is for RESEARCH USE ONLY. DO NOT use for diagnostic and medical purpose.
2. If the package is opened when you have received CardioDISC™ from your selling agent, you should tell it them as soon as possible.
3. CardioDISC™ is derived from BSE-free material, and treated with elimination of bovine antigen, bacteria, and virus. However, the product could contain little or unknown pathogen. Treat the product with wearing gloves to avoid the direct contact.
4. CardioDISC™ should be handled by or accompanying with skilled specialists.
5. CardioDISC™ could be elastic and vent according to the usage condition on account of its contractibility.

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For more information on this product, kindly contact us via email.