Blood response to Arg-Glu-Asp-Val peptide-modified acellular graft

Atsushi Mahara, Tetsuji Yamaoka
Department of Biomedical Engineering, National Cerebral and Cardiovascular Center Research Institute

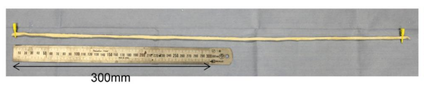
Introduction

REDV peptide on the acellular graft suppresses thrombosis formation and captures blood circulating cells

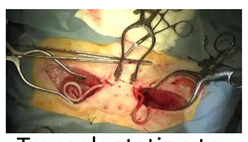
REDV-peptide modified acellular grafts were developed for small diameter long-bypass graft [1,2]. When the grafts with the length of 20-30 cm and the diameter of 2 mm were transplanted to the femoral artery as a bypass graft, the grafts were patent without thrombus formation. From these experiments, we found that thrombus suppression and cell capturing on the graft surface would have contributed to the graft patency.

To prove this concept, we developed peptide-conjugated silane coupling agents (PCSi) for modifying the acellular graft in the peptide density-controlled manner in this study. The REDV immobilized acellular tissue with various REDV densities was prepared using PCSi, and endothelial cell adhesion and platelet adhesion were evaluated *in vitro* and *ex vivo* experiments.

References:
1. Mahara A. et al. *Biomaterials*, 58, 54, 2015.
2. Yamanaka H. et al. *Biomaterials*, 179, 156, 2018.
3. Mahara A. et al. *ACS Biomater. Sci. Eng*, 6, 2050, 2020.

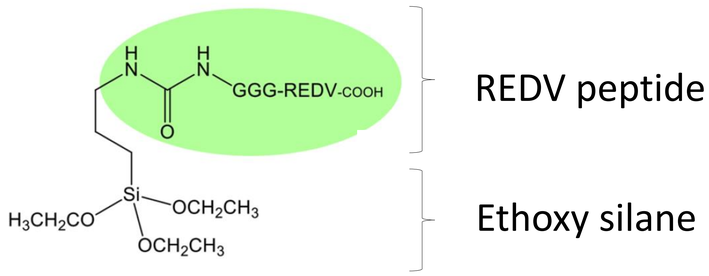


REDV-modified acellular graft
(ϕ2.0-4.0 mm, L=90 cm)



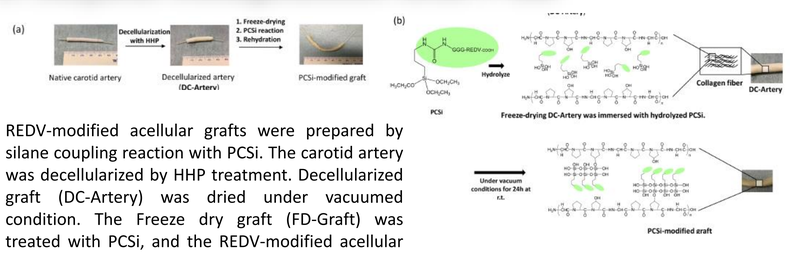
Transplantation to minipig bypass model

Peptide-Conjugated Silane Coupling Agents (PCSi)



Results and Discussion

1. Surface modification with PCSi



REDV-modified acellular grafts were prepared by silane coupling reaction with PCSi. The carotid artery was decellularized by HHP treatment. Decellularized graft (DC-Artery) was dried under vacuumed condition. The Freeze dry graft (FD-Graft) was treated with PCSi, and the REDV-modified acellular graft was acquired.

Figure 1. Preparation of peptide-modified acellular graft by PCSi. (a) Gross images of the native carotid artery, decellularized artery (DC-Artery),and PCSi-modified graft. (b) Modification process of peptide-modified acellular graft by silane coupling reaction of PCSi. HHP; high hydrostatic pressure treatment.

2. REDV peptide-density

Figure 2(a):
Peptide modification and modification area were evaluated by using fluorescence-labeled PCSi. The fluorescence signal was observed in the luminal and medium layer of the graft.

Figure 2(b):
The peptide density was quantified. The density was controlled by the feed concentration of PCSi. The density was ranged from 0.89×10^{-4} to 100×10^{-4} molecules / nm^3.

Abbreviation
DC-graft: Decellularize graft
FD-graft: Freezing dried DC-graft
REDV(X)-graft: PCSi-modified FC-graft
(X; REDV peptide density (x 10^{-4} molecules /nm^3))

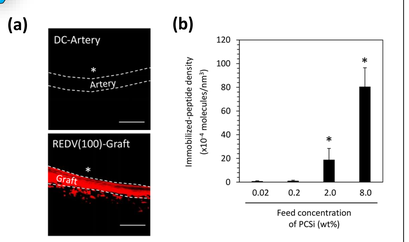


Figure 2. Peptide modification of acellular graft with PCSi. (a) Fluorescence cross-sectional images of DC-Artery and REDV(100)-Graft. White dotted lines indicate the boundary of the tissue. Asterisks indicate the luminal side. Scale bars indicate 200 μm. (b) Immobilized peptide density in PCSi-modified grafts was plotted. (*: p<0.05, n = 5)

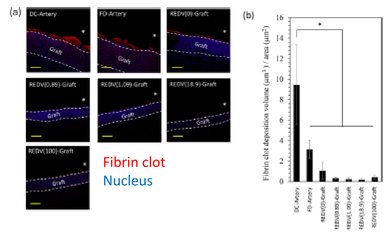
3. Ex vivo Evaluation

Anti-thrombosis property

Thrombus was not observed on the REDV-modified acellular graft, although the surface without the REDV modification was covered with the thrombus.

↓

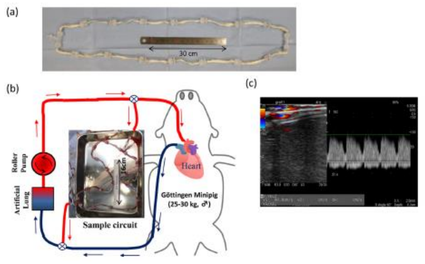
Thrombus adhesion was suppressed independent of the peptide density.



***Ex vivo* evaluation of fibrin clot deposition on the surface of DC-Artery, FD-artery, and PCSi-modified graft for 1 h.** (a) Fluorescence images indicate the cross section of the graft. Scale bars indicate 200 μm. (b) Fibrin deposition volume.

3. Ex vivo Evaluation

The PCSi-modified grafts were connected to the following blood circuit system, and blood was circulated during 1, 3, and 18 hours.

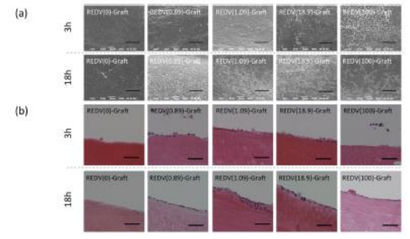


Ex vivo evaluation of blood response to luminal surface of the grafts using a cardiopulmonary bypass circuit system. (a) Eight grafts were connected directly, and the sample circuits with 16 grafts were constructed by connecting two lines in parallel. (b) Cardiopulmonary bypass circuit system connected with the sample circuit. Arterial and venous lines were inserted to the right ventricle and ascending aorta, respectively. The blood flow was controlled by a roller pump. Circulating blood temperature was controlled as 37 ° C by heat exchanger. (c) Blood flow and the velocity at the sample circuit were monitored by echocardiography.

Cell capturing

The cells were observed on the REDV modified surface with increasing the peptide density and blood flowing time.

Blood circulating cells were captured by REDV peptide.



***Ex vivo* evaluation of fibrin clot deposition on the surface of DC-Artery, FD-artery, and PCSi-modified graft for 1 h.** (a) Fluorescence images indicate the cross section of the graft. Scale bars indicate 200 μm. (b) Fibrin deposition volume.

Conclusion

We developed the peptide-conjugated silane coupling agents for a surface modifier of acellular tissues, and REDV-peptide density-controlled acellular grafts were prepared for evaluation in an ex vivo blood response study. We successfully showed cell capture and suppression of fibrin clot deposition on the REDV-immobilized surface under ex vivo blood perfusion conditions. To the best of the authors' knowledge, our finding is the first to show the blood response on an REDV-immobilized surface, and the data are important for the future development of blood-contacting implantable acellular devices.

This work was supported by the Intramural Research Fund of the National Cerebral and Cardiovascular Center (22-2-4) and S-Innovation Project of Japan Agency for Medical Research and Development (AMED).