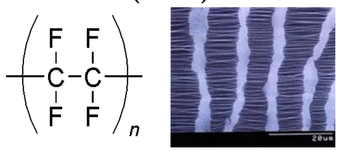
Heparin-Integrin Ligand Co-immobilized Surface for Enhancing the Adhesion of Mesenchymal Stem Cells

○Ryo Minami[1] , Yuki Arichi[1] , Sachiro Kakinoki[1,2]

[1]Department of Chemistry and Materials Engineering, Faculty of Chemistry, Materials and Bioengineering, Kansai University, Osaka, Japan, [2]ORDIST, Kansai University, Osaka, Japan

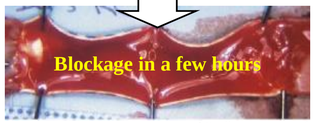
Introduction

Expanded polytetrafluoroethylene (ePTFE)



Anti-thrombogenicity, flexibility, Non-bioabsorbable
➡ ePTFE is widely used for artificial blood vessels.

Transplantation into dog carotid artery



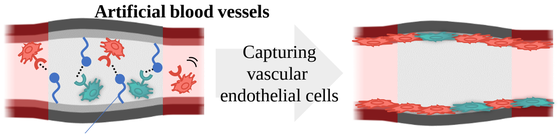
http://www.gore.com/

Factor

- **Inadequate anti-coagulant property**
- **Incomplete pseudo-endothelium**
- **Infiltration and thickening of surrounding connective tissue**
- **Low kinkiness**

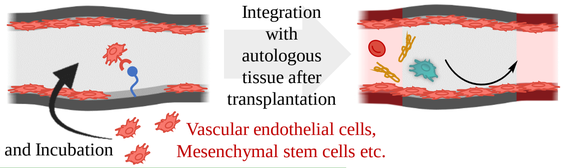
How to prevent blockage of artificial blood vessels? ➡ **Luminal surface of ePTFE should be covered by the stable endothelium tissue rapidly.**

1 ***In situ endothelialization***



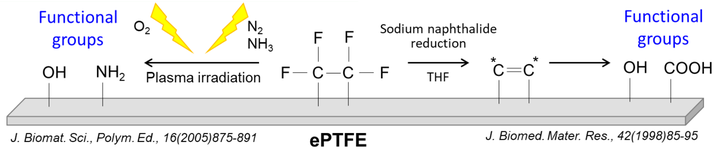
○ No cell transplantation
× Capture Non-target cells

2 ***Ex vivo endothelialization***



○ No contamination with non-targeted cells
○ Intima is matured at the time of implantation.
× Cell transplantation

Strong adhesion of cells on ePTFE with endothelial component cells ➡ **Immobilization of functional molecules on ePTFE**

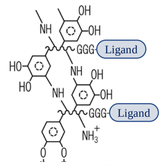


Conventional procedures for ePTFE modification are not suitable for artificial blood vessels because inherent properties of ePTFE will be lost with defluoridation process.
➡ **Simple procedure for the direct immobilization of ligand molecules on ePTFE**

Objective

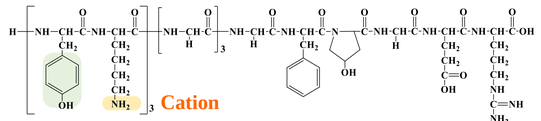
Stable immobilization of ligand peptides and heparin on aminated substrate surface by cross-linking LbL method

Cross-linking by oxidation

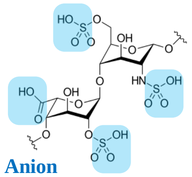


Covalent bonds between quinones and amino groups etc.

S. Kakinoki and T. Yamaoka, Bioconj. Chem., 26(2015)639
S. Kakinoki and T. Yamaoka, JP patent 6041132 (2016)
S. Kakinoki et al., J.Biomed.Mater.Res.A,106A(2018)491



Integrin α_{11} ligand peptide
(Tyr-Lys)$_3$-Gly$_3$-Gly-Phe-Hyp-Gly-Glu-Arg [YK-GER]

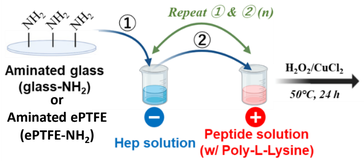


Heparin[HEP]

Layer-by-Layer (LbL)

Formation of adsorption layer by electrostatic interaction between cation and anion

Materials and methods



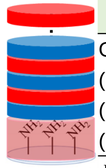


Table 1. Details of the samples used in this study.

Sample	Cation layer	Top layer	Oxidant (H_2O_2 and $CuCl_2$)	LbL cycle
Glass-NH_2	-	-	×	-
$(HEP/YK\text{-}GER)_5$	YK-GER/PLL	YK-GER	×	5
$(HEP/YK\text{-}GER)_5$-ox	YK-GER/PLL	YK-GER	○	5
$(HEP/PLL)_4$-HEP-ox	PLL	HEP	○	4

PLL: Poly-L-Lysine mAdMSC: mouse adipose tissue-derived mesenchymal stem cell

Surface analysis
- **Water contact angle**
- **X-ray photoelectron spectroscopy (XPS)**

***in vitro* functional evaluation**
- **Adhesion of mAdMSC**
- **Adhesion of platelets**
- **Blood coagulation**

Results and discussion

Analysis of substrate surface after LbL and oxidation reaction

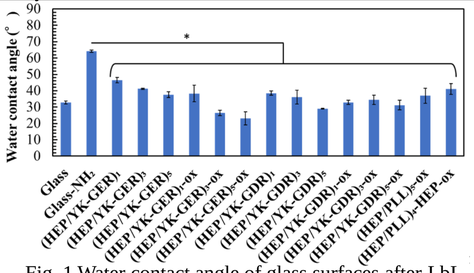


Fig. 1 Water contact angle of glass surfaces after LbL with Hep/YK-ligand and Tyr oxidation.
(GDR: negative ligand sequence)

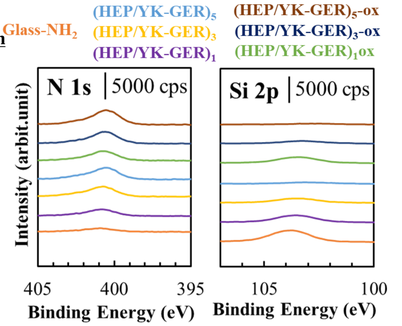


Fig. 2 XPS spectra of glass surfaces.

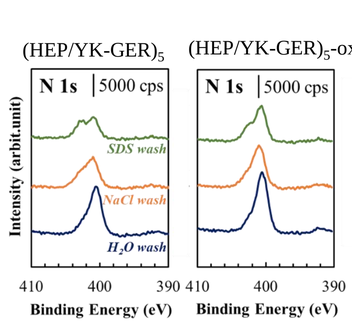


Fig. 3 XPS spectra of glass surfaces after washing by 1% SDS or 1 M NaCl aq.

After $(HEP/YK\text{-}GER)_5$-ox treatment, water contact angle of the glass surface was decreased to 23º and the N1s peaks derived from heparin and peptides were detected by XPS. The adsorbed layer was stabilized by oxidation treatment (ox).

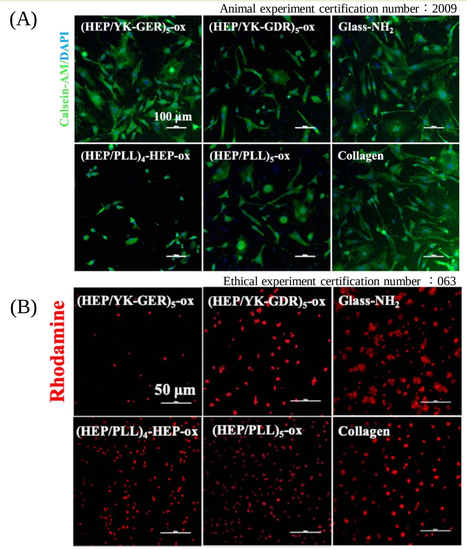


Fig. 4 Adhesion of (A) mAdMSCs and (B) human platelets after 24 and 2 hours incubation, respectively.

$$\text{Blood coagulation rate (\%)} = \frac{\text{Abs (sample)} - \text{Abs (water)}}{\text{Abs (blood)}} \times 100 \quad (\lambda = 576\ \text{nm})$$

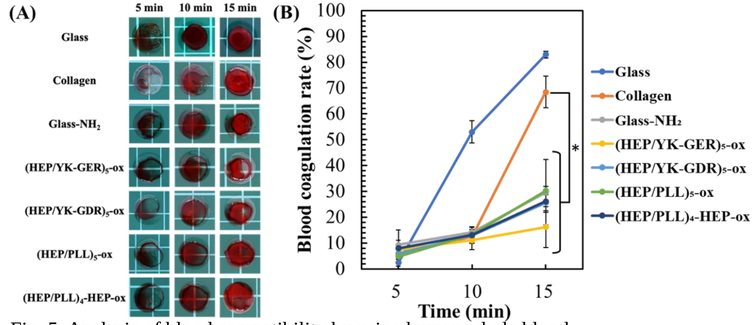


Fig. 5 Analysis of blood compatibility by using human whole blood.
(A) Observation of coagulation behavior and (B) coagulation rate on samples.

The $(HEP/YK\text{-}GER)_5$-ox treatment promoted the adhesion and expantion of mAdMSCs even though it suppressed platelet adhesion and blood coagulation. On the collagen-coated surface, mAdMSCs and platelets adhered, and the blood coagulation was promoted.

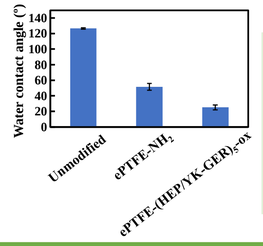


Fig. 6
Water contact angle of ePTFE surfaces after LbL with HEP/YK-GER with Tyr oxidation.

Water contact angle was decreased by $(HEP/YK\text{-}GER)_5$-ox treatment, resulting that the stable LbL layer of HEP/YK-GER was formed not only on glass but also on ePTFE.

Conclusion

Coimmobization of integrin α_{11} ligand peptide and heparin on aminated surfaces was succeeded by the LbL procedure with cross-linking, resulting that AdMSC adhesion and hemocompatibility were promoted.
In the future, we are going to apply this procedure for *ex vivo* endothelialization of ePTFE vascular graft.