Single step immobilization of REDV peptide onto a variety of biomaterial substrates by tyrosine oxidation

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Introduction: Tissue compatibility of prostheses and scaffolds is important in tissue engineering. Because the most of synthetic biomaterials are not biologically active, immobilization of peptides which were isolated from extracellular matrix proteins has been one of the powerful strategies for the design and functionalization of biomaterial substrates. For example, biomimetic surface with Arg-Gly-Asp-Ser peptide can provide cellular adhesiveness. However, the method for peptide immobilization was restricted, and the reactive groups should be introduced on substrates before the peptides immobilization. P. B. Messersmith reported the useful immobilization method of functional molecules including peptides with dopamine and 3,4-dihydroxyphenylalanine (DOPA) on a variety of materials [1]. This method is imitative the adhesive protein secreted by marine mussel and utilizes the high reactivity of catechol group. A problem with this method is the difficulty in the synthesis of DOPA derivatives. In this report, we immobilized peptides onto a variety of biomaterials using single-step oxidation of tyrosine (Tyr) to quinone using hydrogen peroxide and copper (II) chloride catalyst, and tried to apply this technique for preparing re-endothelialization promoting vascular stent.

Methods: REDV peptide containing a Tyr residue (Ac-TG_3REDV) was synthesized by typical Fmoc solid phase procedure and purified by RF-HPLC. Specimens of glass, tissue culture polystyrene (TCPS), polyester (Cell Desk, Sumitomo Bakelite Co., Ltd.) (PESr), tissue culture poly (vinyl chloride) (PVC), expanded poly (tetrafluoroethylene) (ePTFE), poly (L-lactic acid) (Mw=160000) (PLLA) and 316L stainless steel (SS316L) were immersed into an aqueous solution of Ac-YG_3REDV, and then H_2O_2 and $CuCl_2$ were added and incubated for 24 hours (Figure 1). After REDV immobilization, surfaces were analyzed by the water contact angle measurement and X-ray photoelectron spectroscopy (XPS). Human umbilical vein endothelial

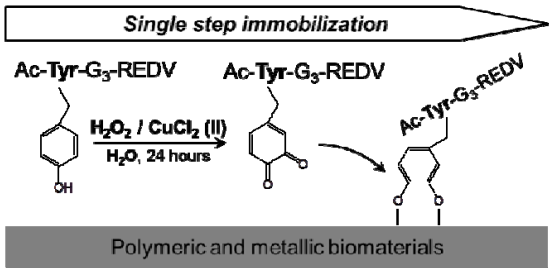

Figure 1. Single step immobilization of REDV peptide onto biomaterials by Tyr oxidation.

cells (HUVEC) were seeded on REDV-immobilized surfaces. In addition, REDV peptide was immobilized on Cr-Co stent as well as SS316L. REDV-immobilize stent was placed into rabbit abdominal aorta, and patency and re-endothelialization in stent were observed after 1 and 6 weeks.

Results: Highly-pure Ac-TG_3REDV was obtained by Fmoc solid phase procedure. After peptide immobilization, water contact angle was decreased and a N1s peak assigned to the peptide was detected in XPS spectra in all substrates. Because these behaviors were not observed in the absence of $CuCl_2$ or H_2O_2, it was suggested that REDV peptide was successfully immobilized onto all substrates by Tyr oxidation. The adhesion of HUVECs was improved on REDV-immobilized surfaces excepting PVC (Figure 2). Because PVC is containing much plasticizer, surface peptide was released with plasticizers by washing. In addition, re-endothelialization was observed at the most part of strut inside REDV-immobilized Co-Cr stent without thrombus formation in a week of stenting.

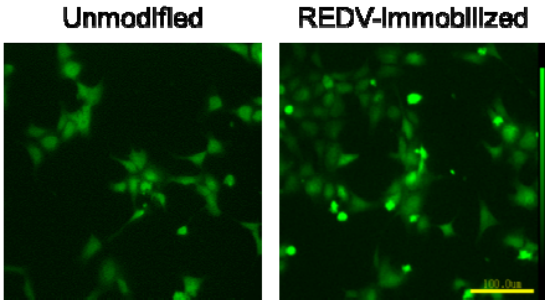

Figure 2. Adhesion of HUVEC on unmodified and REDV-immobilized glass surfaces. (Scale bar; 100 μm)

Conclusions: In this study, we successfully immobilized REDV peptide onto a variety biomaterial substrates by single-step Tyr oxidation. This is the first report to bind natural peptides containing Tyr on biomaterials by a single-step mild reaction. HUVEC adhesion was enhanced on REDV-immobilized surfaces. In addition, re-endothelialization was promoted in REDV-immobilized Co-Cr stent placed into rabbit abdominal aorta. Results of this study demonstrated that our peptide immobilization technique is very useful to design biofunctional medical devices for accelerating in situ re-endothelialization.

References:

1. Lee H. et al., . Science. 2007: 318: 426-430.