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ANTITHROMBOTIC ACTIVITY AND SURFACE CHARACTERISTICS OF LUMBROKINASE-IMMOBILIZED POLYURETHANE

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Lumbrokinase (LK), a strong and novel fibrinolytic enzymes, was extracted from the earthworm, *Lumbricus rubellus* by Mihara et al. These enzymes were very stable and showed greater antithrombotic activity than that of currently-used antithrombotics. We immobilized a LK fraction showing the most potent fibrinolytic activity on polyurethane (PU) surface to investigate its enzymatic and antithrombotic activities. The methanol extracted PU surface was treated with 3% (w/v) maleic anhydride methylvinyl ether copolymer (MAMEC) solution and finally incubated in a LK solution in PBS (pH 7.4). The immobilized LK activity was estimated by the fibrin plate method and caseinolytic activity assay. The antithrombotic activity was evaluated by *in vitro* ^{125}I-fibrinogen adsorption in fresh whole blood and ^{99m}Tc-platelet adhesion tests. The distribution of radiolabelled fibrinogen and platelet was visualized by autoradiography. The occlusion time was also determined through *ex vivo* rabbit A-A shunt. The content and the unit activity of immobilized LK were 8 - 24 $\mu g/cm^2$ and 4 - 18 U/cm^2, respectively. The relative activity ratio of immobilized LK to the soluble LK was about 30%. Immobilized LK was stable over a various pH range and resistant against inhibitors and thermal inactivation. Less fibrinogen was adsorbed and less platelet was adhered on LK-immobilized surface than on PU and PU-MAMEC controls. The *ex vivo* occlusion time of untreated PU and PU-MAMEC surface were only 32 and 42 min, respectively. But that of LK-immobilized PU was extended to 140 min. These results suggested that the immobilized LK on PU surface decreased fibrinogen adsorption by digesting the adsorbed fibrinogen and inhibited platelet adhesion/aggregation to the artificial surface as well, indicating that the LK-immobilized surface was highly antithrombogenic.