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Immobilization of Heparin onto the Polyurethane

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While mechanical properties of using of cardiovascular devices have been overcome, blood compatible remains unsolved. Many researchers have studied that heparin, heparin-albumin complexes, and various fractions of heparin have been dispersed within[1], absorbed[2], ionically bound[3~5], and covalently bound[6~11] to biomaterials to make blood compatible materials. But especially thrombosis has limited the success of vascular grafts with internal diameters of less than 5 mm which are used for the artificial blood vessel. Still no satisfactory blood vessel replacement currently exists. Among them, the most promising materials seem to be certain polyurethane[12, 13].

In this study, we tried to immobilize heparin onto the polyurethane surface by using new reaction scheme. This covalently immobilized heparin onto the biomaterial which provides a stable bond while not altering heparin's ability to interact with a variety of coagulation factors.

Polyurethane (biomer graft) (3.0 mm I.D. size 3) was kindly provided by Thoratec Laboratories Corporation, Berkeley, CA 94710, U.S.A., Polydisperse sodium heparin, activity −160 U/mg, used for immobilizations, was of the porcine intestinal mucosal variety (Diosynth, Inc.). Other chemicals were used as chemical grade without further purification. The toluidine blue method, described by Smith[14], was used to find resulting heparin surface concentration. Heparin was immobilized via a spacer arm onto the biomer graft through a 4-step reaction scheme (Fig. 1).

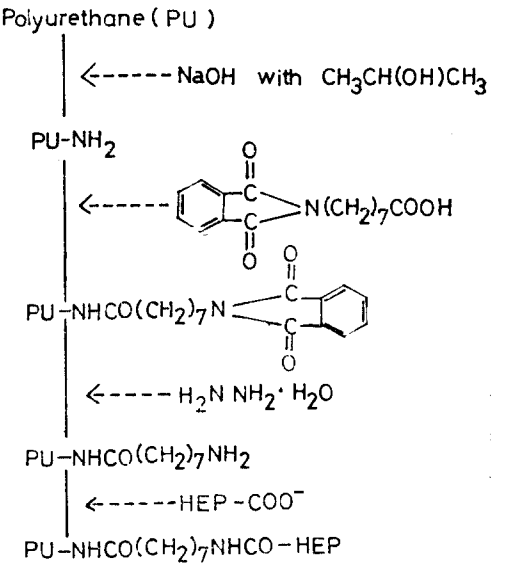

Fig. 1. Reaction scheme for immobilization of heparin onto the polyurethane

Hydrolysis of biomer graft was performed in 3.0 M NaOH for 6 hrs at 78°C in 10% isopropanol (volume) as swelling agent.

This created free amine and hydroxy groups.

The amino group of the spacer group (am-

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ino carboxylic acid: $NH_2(CH_2)_nCOOH$ n=2~11) was blocked with N-carboethoxyphthalimide. e.g: To 5 ml of water blocked with N-carboethoxyphthalimide. w-aminocaprylic acid [$NH_2(CH_2)_7COOH$], 575mg(2nm) sodium carbonate and 450 mg(2.1 mM) of N-carboethoxy phthalimide were added. The mixture was stirred for about 30 min. The solution was then filtered and the filtrated acidified. After crystallization from water and drying, phtaloyl amino caprylic acid was obtained.

72.3 mg(2.5×10^{-4}M) of the blocked spacer in 10 ml of ethanol was attached to the biomer graft with 50.9 mg(2.5×10^{-4}) of N, N'-dicyclohexylcarbodiimide as an activating at room temperature for 24 hrs.

The attached spacer to the biomer graft was then deblocked in a 1.0 M hydrazine hydrate in ethanol solution at room temperature for 24 hrs.

A total of 480 mg of 1-ethyl-3-(3-dimethylamino propyl) carbodiimide hydrochloride as an activating agent in 10 ml water was added to the 1.0 g of heparin in 15 ml water in approximately 10 mg portions over a 6 hr period while the reaction was maintained at 4°C and pH 4.75. A 25 ml aliquot of the activated heparin solution was added to the amino caprylic-derivatized biomer graft, the resultant materials being gently stirred at room temperature for 24 hrs.

The heparin immobilized surface was assayed for heparin content with a chromogenic assay of toluidine blue. It has been calculated that the surface content of heparin is approximately 4 μg per cm^2.

In vitro and vivo studies are currently being undertaken.

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