쥐의 피하조직에 이식된 후 설폰산화 폴리에틸렌옥사이드가 결합된 폴리우레탄의 생체내 안정성과 항칼슘화 특성

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In vivo biostability and calcification-resistance of sulfonated polyethyleneoxide-grafted polyurethane after subcutaneous implantation in rats

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I. INTRODUCTION

Biomedical polymers for surgical implants require outstanding physical and mechanical properties, stability in vivo, nontoxicity, sterilizability and biocompatibility. Among these characteristics, biostability in vivo is the most crucial factor in long-term performance of implanted biomaterials. Although polyurethanes(PUs) are widely used in biomedical devices due to their superior physical properties and relatively good biocompatibility(1), recently, in vitro and in vivo degradation of PUs are reported by some investigators(2-4). The possible degradation hypotheses are as follows: a) oxidation by oxygen and hydrogen peroxide, b) hydrolysis by enzymatic attack, c) environmental stress cracking, and d) calcification by calcium deposition.

In particular, the calcification that is defined as the deposition of calcium compounds such as either some calcium phosphate minerals consisting of hydroxyapatite or the calcium salts results from loss of the flexibility of biomaterials, thereby causing their mechanical failure and degradation(5). It is known that implanted medical devices lead to the most dystrophic calcification, where the tissues are necrotic or otherwise altered in normocalcemic subjects. Such a calcification associated with biomaterials occurs very often in bioprosthetic heart valves, aortic homografts, and polymeric blood pumps and trileaflet valves and also frequently in mechanical heart valves, vascular grafts, and subcutaneous implanted materials in rodents(6).

Therefore, in order to be utilized to modified PUs with long-term stability for artificial organs, especially the cardiovascular system of artificial heart and its valves, the features of anticalcification as well as blood compatibility in the implants are demanded.

In our previous studies, PUs grafted by hydrophilic polyethyleneoxide (PEO) (PU-PEO) and further sulfonated by propane sultone (PU-PEO-SO_3) or by hydrophobic perfluorodecanoic acid (PFDA) (PU-PFDA) have showed enhanced thromboresistance(7-9). In this work, as calcification is closely related to antithrombogenicity, in vivo rat subcutaneous implantation is performed by using these blood compatible PUs to clarity the mechanism of calcification and to examine in vivo biostable and calcification-resistant polymer.

II. MATERIALS AND METHODS

Surface Modification of PUs

The methods of surface modification of highly hydrophobic PU-PFDA, hydrophilic PU-PEO1000, and negatively charged PU-PEO1000-SO_3 have been previously described in detail, as shown in Fig. 1(9-11).

The surface of PU sheet was treated with HMDI to introduce free isocyanate (-NCO) groups in toluene with stannous octoate at 40 ℃ for 1h under nitrogen (PU-HMDI). Consecutively, PU-HMDI was reacted with PFDA in toluene for 2 h at 40 ℃ to obtain PU-PFDA. Meanwhile, PEO1000 was also grafted onto PU-HMDI in benzene with stannous octoate for 24 h at 40 ℃ to yield PU-PEO1000. The hydroxyl end groups of grafted PEO1000 chains were further sulfonated by PST in a mixture of isopropanol, sodium carbonate, and dimethyl sulfoxide for 20 h at 45 ℃ to produce PU-PEO1000-SO_3.

In Vivo Animal Test

Animal study for the biostability and calcification of modified PUs was conducted by subcutaneous implantation in male rats (60-80g). The rats were anesthetized with ketamine, and the abdominal area was shaved, cleaned with alcohol and swabbed with iodine. A mid-line incision was made in the skin, which was then gently dissected away from the abdominal muscles. A small intra-muscular pouch was made and 1 x 1 cm specimen sheet inserted in the pouch.

Two such implants were done on each side of the mid-line, for a total of 4 implants of the different material surface in each animal. The skin incision was closed with the suture. The rats were sacrificed at 2, 4, and 6 months after implantation by an overdose of ketamine, and the implants were retrieved.

Characterization of Biostability and Calcification

The surface morphology and the existence of calcium and phosphorus atoms of retrieved sheets were examined with energy dispersive X-ray analysis (EDXA, Kevex Delta IV) having the quantum Si detector, coupled with a scanning electron microscopy (SEM, Hitachi 2500C). Specimens were mounted and sputter-coated with carbon using an ion coater and observed at an accelerating voltage of 20 kV.

The quantitative analysis of calcium and phosphorus deposited on the sheets was carried out as follows: the retrieved sheets was slightly rinsed with the deionized water and extracted individually with shaking for 5 days at 65 ℃ in 0.6N HCl of 5 ml. Then, these extract solutions were assayed by inductively coupled plasma atomic emission spectrometer (ICP, Plasmascan 710, Lattam Co.).

Tissue ingrowth and calcification on the sheets were evaluated from implant material-tissue reactions. The implanted sheets were removed with surrounding muscle tissue, fixed in neutral buffered formalin, dehydrated with a series of ethanol solution, and imbedded in glycolmethacrylate medium for histological preparation and light microscopy. Sections cutted by microtome were stained with hematoxylin and eosin for overall tissue reaction, and other sections were also stained by von Kossa's method for calcium.

III. RESULTS AND DISCUSSION

From the SEM observation on sample surface removed from rats the degree of crack formation was increased in the following order: PU-PFDA = PU > PU-PEO1000 > PU-PEO1000-SO_3. This result means that PU-PEO1000-SO_3 is the most biostable in vivo. Generally, cracks have been known to act as nucleation sites for thrombus formation or calcification so that they enfeeble the material. Also, because calcification has considerable connection with the surface roughness, even though PU-PFDA show improved blood compatibility, it is thought that textured rough PU-PFDA is not good for anticalcification material.

In results of EDXA and quantitative analyses of calcium and phosphorus by ICP, the deposition of calcium was found abundantly but the phosphorus was hardly in existence (Table 1), suggesting that this calcium compound is not a hydroxyapatite. The deposition amount of calcium after implantation for 6 months was also orderly PU-PFDA > PU > PU-PEO1000 > PU-PEO1000-SO_3 (Fig. 2).

Meanwhile, The degree of tissue response varies according to both the physical and the chemical nature of the implants. All the implants did not provoke an intense tissue(host) reaction, but they were walled off from the muscle by a fibrous capsule, where fibrocytes were matured and collagen was laid down. Especially, PU and PU-PFDA have shown that some inflammatory cells such as lymphocyte and chronic inflammatory cells was still infiltrated to the surrounding collagenous tissue including slight regeneration of muscle. Also, calcium depositions observed by von Kossa staining was seen in a blood vessel within the connective tissue in the case of PU and PU-PFDA.

Therefore, PU-PEO1000-SO_3 is promising as calcification-resistant and in vivo biostable polymer, useful for blood contacting device materials.

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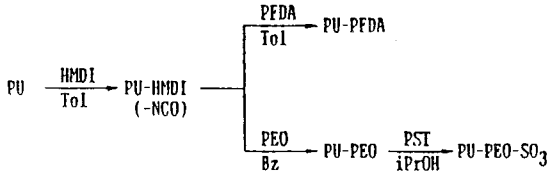

Fig. 1. Modification scheme of PU surfaces.
HMDI = $OCN(CH_2)_6NCO$, PFDA = $CF_3(CF_2)_8COOH$,
PEO = $HO(CH_2CH_2O)_nH$, PST = $(CH_2)_3SO_3$

Table 1. The contents[a] of calcium and phosphorus on implantation time

Material	Ca			P		
	2[b]	4	6	2	4	6
PU	79 ± 24	154 ± 43	221 ± 43	0.71 ± 0.46	0.30 ± 0.13	0.37 ± 0.09
PU-PFDA	155 ± 29	261 ± 61	348 ± 57	0.86 ± 0.43	0.33 ± 0.09	0.41 ± 0.15
PU-PEO1000	66 ± 7	120 ± 27	162 ± 35	0.58 ± 0.18	0.15 ± 0.07	0.29 ± 0.07
PU-PEO1000-SO_3	57 ± 17	71 ± 24	93 ± 24	0.52 ± 0.04	0.09 ± 0.04	0.27 ± 0.05

a. Unit: $\mu g/cm^2$, Mean ± S.D.(n = 5 - 7)

b. Unit : months

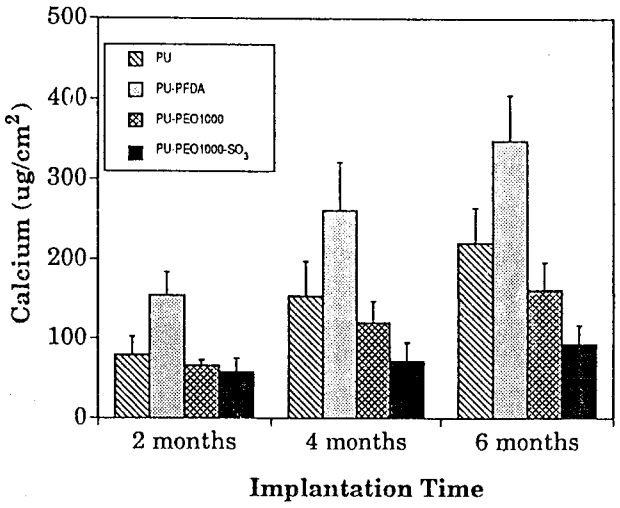

Fig. 2. Calcium concentration deposited on modified PUs.