생체적합성 PU-PEO-SO_3 재료의 *In Vivo* 동물실험: 수채형 양엽 고분자심장판막 및 인공혈관으로의 응용

한동근° · 이규백* · 박기동 · 정서영 · 김영하 · 김철생* · 김형묵** · 민병구*, 한국과학기술연구원 고분자설계실, *서울의대 의공학과, **건국의대 의학공학과, ***고려의대 흉부외과

In Vivo Canine Studies of Biocmpatible PU-PEO-SO_3: Application for Sinkhole Bileaflet Polymer Heart Valve and Vascular Graft

D.K. Han° , K.B. Lee*, K.D. Park, S.Y. Jeong, Y.H. Kim, C.S. Kim**, H.M. Kim***, and B.G. Min*, Polym. Chem. Lab., KIST, Dept. of Biomed. Eng., *Seoul National Univ., **Kon Kuk Univ., ***Dept. of Thorac. Surg., Korea Univ.

INTRODUCTION

Polyurethane (PU) has been widely used in biomedical devices due to its superior mechanical properties, however its blood compatibility is not satisfactory yet (1). Recently, many studies have been reported on biostability of PU during long-term implantation. Factors affecting degradation of PU involve primarily calcification, oxidation, hydrolysis, and absorption of lipid resulting in environmental stress cracking (2). The calcification defined as the deposition of calcium compounds such as either some calcium phosphate minerals consisting of hydroxyapatite or the calcium salts, is thought to have considerable connection with both the physical and the chemical nature of the implants. The calcification causes the loss of the flexibility of biomaterials, resulting in their mechanical failure and degradation (3). Therefore, it is required that biomedical polymers for long-term implantation have blood compatible, biostable, and calcification-resistant characteristics.

In our previous studies (4,5), sulfonated poly(ethyleneoxide) (PEO)-grafted PU (PU-PEO-SO_3) was shown to improve *in vitro* and *ex vivo* blood compatibility, *in vivo* biostability and anticalcification due to the synergistic effect of the nonadhesive and mobile PEO chains and negative charged sulfonate (SO_3) groups.

In this study, to examine *in vivo* performance and biocompatibility of a newly designed Sinkhole valve and vascular graft for total artificial heart (TAH) and ventricular assist devices (VAD) and to further investigate the correlation between blood compatibility and biostability as well as anticalcification of implanted polymers, PU-PEO-SO_3 was applied as a coating material over the polymer heart valve and vascular graft and its biological responses were evaluated using *in vivo* canine ventriculo-pulmonary shunt system.

MATERIALS AND METHODS

Synthesis of PU-PEO-SO_3

The preparation of PU-PEO-SO_3 by bulk modification is as follows (6): First, the reaction of amino-terminated PEO (MW=1000) with propane sultone is performed at 50 ℃ for 5 hrs to get sulfonated PEO (H_2N-PEO-SO_3). Subsequently, H_2N-PEO-SO_3 is reacted with hexamethylene diisocyanate (HMDI) at 50 ℃ for 3 hrs to yield isocyanated PEO-SO_3 (OCN-PEO-SO_3). Finally, OCN-PEO-SO_3 is grafted to PU (Pellethane 2363-80AE; Dow Chemical Co.) dissolved in dimethylacetamide (DMAc) for 3 days at 50 ℃ to produce PU-PEO-SO_3.

The obtained PU-PEO-SO_3 was dissolved in DMAc (2.5 %, w/v) and coated on Sinkhole bileaflet polymer heart valve and porous curved vascular graft. Untreated PU was used as a control.

Fabrication of Sinkhole valve and vascular graft

Figure 1(a) illustrates the schematic diagram of the Sinkhole bileaflet polymer heart valve. The detailed methods on fabrication of Sinkhole valve have been described previously elsewhere (7). It was newly designed and prepared with PU in order to prevent the prolapse of leaflet and to maximize the effective orifice area, compared with the existing bileaflet polymer valves.

Figure 1(b) shows schematic diagram of right ventricle (RV)-pulmonary artery (PA) shunt connected with a curved PU graft. Porous curved large-diameter PU vascular graft is fabricated as follows (6). The curved clean glass mold (19 mm in diameter, half-circle, 5 cm in curvature) was dipped into 24 % (w/v) PU solution in DMAc and then immersed into the 30 % (v/v) ethylene glycol/ethanol solution to give porosity to

prepare PU vascular graft.

In vivo animal test

In vivo canine studies for a newly designed Sinkhole valve and porous curved vascular graft were performed by shunt method between RV and PA (Fig. 1b). Before implantation, Sinkhole valve leaflet and vascular graft were treated by half-and-half coating with each 2.5 % (w/v) PU and PU-PEO-SO_3 solution in DMAc. The procedure for animal test is reported in detail elsewhere (8).

Characterization of biocompatibility

Gross and SEM observation

The thrombus and crack formation and surface morphology on retrieved samples were examined by gross observation and scanning electron microscopy (SEM, Hitachi 2500C). Samples for SEM were mounted and sputter-coated with gold using an ion coater and observed at an accelerating voltage of 15 kV.

Calcium analysis

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

RESULTS AND DISCUSSION

In vivo performance

Table 1 lists *in vivo* performance of Sinkhole valve and vascular graft. The survival periods in 3 cases of implantation were 14, 24, and 39 days, respectively. Each dog died of infection, pneumothorax, and hemothorax, respectively, regardless of their malfunction. No mechanical failure was observed in each Sinkhole valve and vascular graft, suggesting that these Sinkhole valve and vascular graft for TAH and VAD were well functioned during implantation. A newly designed Sinkhole bileaflet polymer heart valve will be feasible for temporary use in blood pumps, and a large-diameter porous PU vascular graft can be utilized for TAH graft and cuff.

Blood compatibility

From explanted samples, no significant thrombus formation was found by gross observation, although some depositions of small thrombi were in the crevice formed between the valve frame and the graft, and inside the untreated PU graft. However, there was no evidence of thrombus formation on the valve leaflet, frame, and PU-PEO-SO_3-coated graft.

SEM studies demonstrated much less platelet adhesion and thrombus formation on PU-PEO-SO_3 than PU in vascular graft. The valve leaflet also exhibited similar trend to vascular graft, independent of implantation time. These results imply that PU-PEO-SO_3 is more blood compatible than PU, as a coating materials in both valve leaflet and vascular graft.

Biostability

From SEM observation, the cracks in valve leaflet were occasionally observed on only PU surface but not on PU-PEO-SO_3. There were some adhesion of blood components in only their vicinity. It suggests that there exists relationship between blood compatibility and biostability in implanted polymers. PU-PEO-SO_3 surface is thought to be more biostable than PU, regardless of both implant position and time.

Anticalcification

Figure 2 shows calcium concentration deposited on implants. The content of calcium deposited on vascular grafts decreased as compared with valve leaflet. This high calcium deposition on valve leaflet may be attributed to its dynamic motion and thereby the concentrated mechanical stress. The degree of calcification on PU-PEO-SO_3 was lower than PU, meaning that PU-PEO-SO_3 is calcification-resistant. In addition, the deposition amount of calcium increased as implantation time increased from 14 days to 39 days regardless of the kind and position of implants.

In summary, the above results attest that PU-PEO-SO_3 is more *in vivo* blood compatible, biostable, and calcification-resistant than untreated PU, as a coating material for polymer valve and vascular grafts in canine implantation. The enhanced blood compatibility, biostability, and anticalcification of PU-PEO-SO_3 is attributed to the synergistic effect of hydrophilic, nonadhesive, and mobile PEO chains and negatively charged sulfonate (SO_3) acid groups (4,5).

Therefore, the blood compatibility of modified PU is closely related to its biostability and anticalcification: the more blood compatible modified PU is, the more biostable (less degradable) and calcification-resistant it is.

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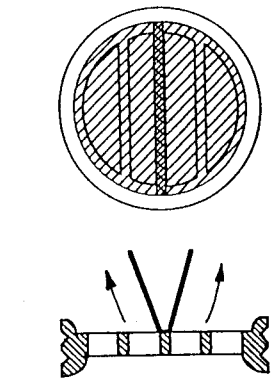

Figure 1. Sinkhole valve and vascular graft: (a) schematic view of Sinkhole valve, (b) schematic diagram of canine RV-PA shunt system.

Table 1 *In vivo* performance of Sinkhole valve and vascular graft

Case	Survival period (days)	Cause of death
I	14	infection
II	24	pneumothorax
III	39	hemothorax

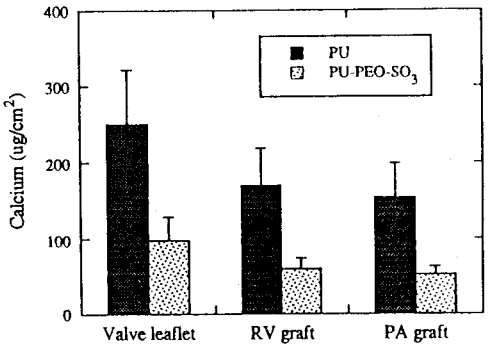

Figure 2. Calcium concentration deposited on various implants after 39 days implantation in dog.