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Biocompatibility and Long-Term Toxicity of InnoPol® Implant, a Biodegradable Polymer Scaffold

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Abstract: *InnoPol®, a poly($_{D,L}$-lactic-co-glycolic acid) [PLGA] 65/35 scaffold manufactured by special gas foaming methods in Korea, was subjected to tests to evaluate the degradation and tissue compatibility characteristics and long-term systemic toxicity in mice and rats. C57BL/6 mice and SD rats were implanted subcutaneously with 3-mm- and 1-mm-thick InnoPol® circular discs, 10 mm in diameter, respectively, and sacrificed 8, 12, and 24 weeks after implantation. No test material-related effects were observed in mortality, clinical signs, body weight gain, food and water consumption, ophthalmologic signs, urinalysis, hematology, serum biochemistry parameters and organ weights of all animals implanted with InnoPol®. Also, there were no systemic symptoms including metabolic alterations and inflammatory reactions in either mice or rats. In addition, no gross pathological findings, except skin lesions around the implantation sites, were found in the major organs. Although mild inflammation at the site of InnoPol® implantation was confirmed from hematoxylin and eosin or Masson's trichrome staining at 8–12 weeks, the reactions had disappeared at 24 weeks following complete degradation of the scaffold, leaving granulomatous tissues that were similar to surgical wounds in sham operation controls without implants. These results suggest that InnoPol® possesses good mechanical properties and tissue compatibility and does not cause any systemic toxicity other than transient local inflammatory reactions at the implantation site, and that it might be useful in applications as a medical device for implantation.*

Key words: *biocompatibility, InnoPol®, poly($_{D,L}$-lactic-co-glycolic acid) [PLGA], scaffold, long-term toxicity*

Introduction

Synthetic biodegradable polymers have been widely utilized as scaffolds in tissue engineering to direct specific cell growth and differentiation [30]. Porous scaffolds with an open pore structure are often desirable in many tissue engineering applications in order to maximize cell seeding, attachment, growth, extracellular

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matrix production, vascularization, and tissue ingrowth. A macroporous structure with uniform pore sizes over 100 μm is highly desirable for efficient cell seeding and culture, to allow for the sufficient nutrients and oxygen to facilitate tissue formation [47].

Biodegradable polymers are of interest in medicine because these polymers produce non-toxic degradation products which ultimately circumvent surgical removal of the drug-depleted devices [1, 4]. So both the use of biomaterials and the number of different applications for biomaterials are increasing. Poly ($_{D,L}$-lactic-*co*-glycolic acid) [PLGA] copolymers are among the few synthetic polymers approved for human clinical uses. They can be easily processed into the desired configuration and their physical, chemical, mechanical, and degradative properties can be engineered to fit a particular need [20]. Porous PLGA foams have been utilized for the regeneration of various tissues and organs such as cartilage, bone, heart, valves, nerves, muscles, bladder, liver, etc. So, polymers have been used as surgical devices for more than 30 years for various medical applications including controlled drug-delivery systems, dental and orthopedic devices, sutures, cardiac pacemakers and vascular grafts [10, 26, 37, 38]. The biocompatibility of PLGA has also been demonstrated in many biological sites [20].

For polymers to be used in biological systems, adequate testing of their tissue biocompatibility is required. Before a biomaterial can be applied clinically, it has to be certified as non-cytotoxic and biocompatible [12]. Ideally, these materials should not elicit any systemic, immunologic, cytotoxic, mutagenic, carcinogenic or teratogenic reactions when implanted *in vivo*. Practically, it would be difficult to find materials that would be completely tissue compatible, since the body recognizes the implant as foreign material and isolates it by encapsulation. Thus, biomaterials described as tissue compatible are often those that show tissue tolerance after chronic exposure [10].

In the case of medical devices for implantation in tissues such as bone and cartilage for more than 30 days, they must be evaluated for biological safety, such as cytotoxicity, sensitization, irritation, genotoxicity, encapsulation in implantation, hemolysis, pyrogenicity, acute systemic toxicity, subacute systemic toxicity, chronic systemic toxicity and carcinogenicity. Generally, in systemic toxicity tests of implants, extract of the implant is applied to animals, not the implant itself. *In vitro* studies represent ideal conditions, which are hardly expected *in vivo*. The real type and concentration of degradation products *in vivo* and the pH surrounding the implants are not fully known. Conditions could be very different depending on the amount of the implanted polymer, the local ability of tissues to clear degradation products, and the degradation of the polymers, which in turn depends on the initial molecular weight, the copolymer composition, the shape and size of the implants, and the mechanical conditions at the implantation site. Therefore, it is difficult to extrapolate *in vitro* results directly to *in vivo* situations. Moreover, *in vitro* results from different studies can be compared only in a restrictive manner, because the test conditions such as, cell lines, exposure to polymers and preparation of extracts vary widely [11].

There are several methods used to fabricate highly porous biodegradable polymer cell scaffolds, including particulate-leaching [23, 24, 33], phase separation [19, 27, 32], gas foaming [25, 28], emulsion freeze drying [42], and 3-D printing techniques [29]. These methods can be used to fabricate a sponge-like scaffold, which then can be laminated into three-dimensional foams [23] or formed into more complex architectures known as superstructures [43]. InnoPol®, a porous PLGA 65/35 scaffold manufactured by special gas foaming methods was introduced by Innotech Med., Co. in Korea. Recently Yoon and Park reported that macroporous biodegradable scaffolds could be successfully fabricated using ammonium bicarbonate salt as a gas foaming agent as well as a porogen additive [47].

In the present study, poly ($_{D,L}$-lactic-*co*-glycolic acid) [PLGA] 65/35 scaffold, named InnoPol®, was subjected to tests to evaluate the degradation and tissue compatibility characteristics *in vivo* and subacute systemic toxicity in C57BL/6 mice and SD rats for 24 weeks. Mice and rats were implanted subcutaneously with InnoPol®, sacrificed at 8, 12, and 24 weeks after implantation and the tissue reactions were histologically evaluated.

Materials and Methods

Scaffold fabrication

Poly (DL-lactic-co-glycolic acid) of lactic/ glycolic molar ratios of 65/35 was purchased from Alkermes

(OH, USA). Weight average molecular weights of PLGA 65/35 were 74,300, as measured by a gel permeation chromatography system. PLGA 65/35 scaffold, InnoPol®, was fabricated and provided by Innotech Medical, Inc. (Daejon, Korea). Viscous polymer precipitated in a state of gel paste was prepared first by dissolving 5 g PLGA in 12 ml of chloroform. Sieved ammonium bicarbonate salt particulates (salt particle size was 300–500 μm) were added to the PLGA gel paste and mixed homogeneously. The weight ratio of NH_4HCO_3 to PLGA was adjusted to 8 : 1. A gel paste mixture of polymer/ nonsolvent/ salt was put into molds (10 × 1 mm and 10 × 3 mm disc type) and the nonsolvent (ethanol) was evaporated at room temperature to obtain a solidified mass. This was immersed into an aqueous citric acid solution of 50% (w/v) concentration at room temperature to induce gas foaming. After the completion of the effervescence, the porous polymeric scaffolds were taken out of the molds, washed with distilled water several times, and then completely dried in a vacuum chamber. Finally, the dried scaffolds were sterilized in 10% EO gas and then packed in an aluminum pouch. There were no abnormalities of the external appearance, such as cracks, cuts, damages and the other defects for the normal use.

Animals and housing

All animal experiments were performed after receiving the approval of the Institutional Animal Care and Use Committee (IACUC) of the Clinical Research Institute of Seoul National University Hospital. National Research Council (NRC) guidelines for the care and use of laboratory animals were observed (revised 1996). Six-week old male C57BL/6 mice were purchased from Charles River Japan, Inc. (Kanagawa, Japan). Six-week old male Sprague-Dawley rats were purchased from Daehan Biolink, Inc. (Chungbuk, Korea). The animals were acclimated to the laboratory for one week. The mice and rats were housed individually in animal rooms with environmentally-controlled temperature (22 ± 2°C), relative humidity (50 ± 10%), air ventilation (12–18 times/h), and a 12-h light/dark cycle. Gamma-ray irradiated laboratory rodents diet (Purina Korea. Co., Kyeonggi, Korea) and autoclaved water were given *ad libitum*.

Implantation of scaffolds

InnoPol® (10 × 1 mm and 10 × 3 mm disc type) was unsealed in a sterilized environment and completely submerged in refrigerated 75% ethanol (at 4°C) for more than five minutes. The scaffold was removed from ethanol and rinsed with cold distilled water 3 times. During the third rinse, the scaffold was placed in a vacuum while submerged in distilled water, to remove the bubbles inside. After rinsing with phosphate-buffered saline (PBS) or saline twice, the scaffold was preserved in cell culture medium.

Animals were anesthetized by intraperitoneal injection with a mixture of ketamine (50–100 mg/kg) and xylazine (5–10 mg/kg). The animals were shaved and sterile-prepped with betadine and alcohol. An incision (approximately 1 cm long) was made on central area of the back using a surgical blade. The prepared scaffold disc was inserted beneath the dermal layer and then the incised skin was sutured using sterile Autoclip. Scaffolds with a size of 10 × 3 mm were implanted to mice and 10 × 1 mm to rats. Animals were given an intramuscular prophylactic dose of gentamicin (25 mg/kg). The animals were sacrificed at specific time points (4, 8, 12 and 24 weeks) after implantation.

Observation

Mortality and clinical signs

All animals were observed for mortality and signs of overt toxicity once daily after implantation throughout the study.

Body weights

Individual body weights were recorded prior to study initiation and weekly during the study.

Food and water consumption

Food and water consumption were measured individually at weekly intervals for 12 weeks, and once every four weeks thereafter. The amounts of food and water were calculated before they were supplied to each cage and their remnants were measured next day to calculate the difference which was regarded as daily food and water consumption (g/animal/day).

Urinalysis

Urine was collected from five animals per group once at 24 weeks after implantation. The parameters deter-

mined in urinalysis included pH, specific gravity, leukocyte, nitrite, protein, ketone body, urobilinogen, bilirubin, glucose and occult blood using an urinalysis stick (N-multistix, Ames, Germany) and an urine analyzer (Miditron Junior II, Roche Co., Germany).

Ophthalmoscopy

An ophthalmologic examination was conducted on five animals per group once at the end of study. Ophthalmologic examinations on the anterior segment of the eye, lens and ocular fundus were performed using an indirect ophthalmoscope (Keeler ALL PUPIL, UK).

Hematology

Blood samples were collected from the posterior vena cava in EDTA-containing tubes under anesthesia. The hematological parameters including white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb) concentration, hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet (PLT) count were examined using an Animal Blood Counter (Vet abc, France). WBC differential counts including lymphocyte, neutrophil, eosinophil, basophil and monocyte were determined from blood smears stained with Wright-Giemsa.

Serum biochemistry

Blood for clinical chemistry was placed in tubes devoid of anticoagulant, allowed to clot at room temperature, and centrifuged, and the serum was separated. The sera were stored at –80°C in a freezer before they were analyzed. Serum biochemistry parameters such as total protein (TP), albumin, A/G ratio, glucose, total cholesterol, triglyceride, total bilirubin, BUN, creatinine, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), Cl, Ca, K and P were analyzed with an automatic chemistry analyzer (HITACHI-7070, Japan). Prothrombin time (PT) and activated partial thromboplastin time (aPTT) were determined using a blood coagulation analyzer (ACL-100, Italy) in plasma samples treated with 3.13% sodium citric acid.

Gross findings at necropsy and organ weights

All surviving animals were sacrificed by exsanguination under anesthesia at the end of the observation period and examined carefully for macroscopic abnormalities. Representative samples of protocol-designated organs and tissues were collected and fixed in phosphate-buffered neutral formalin. The major organs and tissues including heart, liver, lung, spleen, kidneys and thymus were weighed. Organ weights from all animals surviving until the scheduled sacrifice were recorded along with postmortem body weights and appropriate weight ratios (absolute and relative to body weights) calculated.

Histopathology

Fixed organs and tissue such as skin, mammary gland, lymph node, salivary gland, femur, bone marrow, thymus, trachea, lung, bronchus, heart, thyroid glands, parathyroid glands, tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, liver, pancreas, spleen, kidney, adrenal glands, urinary bladder, seminal vesicle, prostate, testes, epididymis, ovaries, uterus, vagina, brain, spinal cord, and eyes were routinely processed, embedded in paraffin and sectioned at 3–5 μm. The sections were stained with hematoxylin-eosin stain for light microscopic examination. The skin tissues adjacent to the scaffold (about 20 × 20 mm) were harvested using a No. 10 surgical blade. The remaining polymer scaffolds with surrounding tissues were fixed in 10% neutral formalin solution for histology. Tissues were trimmed, routinely processed, paraffin embedded, and sectioned. The sections were stained with hematoxylin & eosin, or Masson's trichrome stain and examined for histology. The tissue response was rated according to the following scoring system: – = no infiltration to ++++ = extensive infiltration of granulocytes, giant cells and lymphocytes. The sections were also examined for the presence of fibrin, exudate, the induction of vascularization and stroma (extracellular matrix with blood vessels and fibroblasts) and the formation of a fibrous capsule around the scaffold as well as possible alterations of the scaffold (Cadee *et al*., 2001).

Statistical analysis

Body weights, food consumption, water consumption, hematology, biochemistry and urinalysis parameters, and absolute and relative organ weights were analyzed using one-way analysis of variance (ANOVA). If ANOVA indicated significant difference

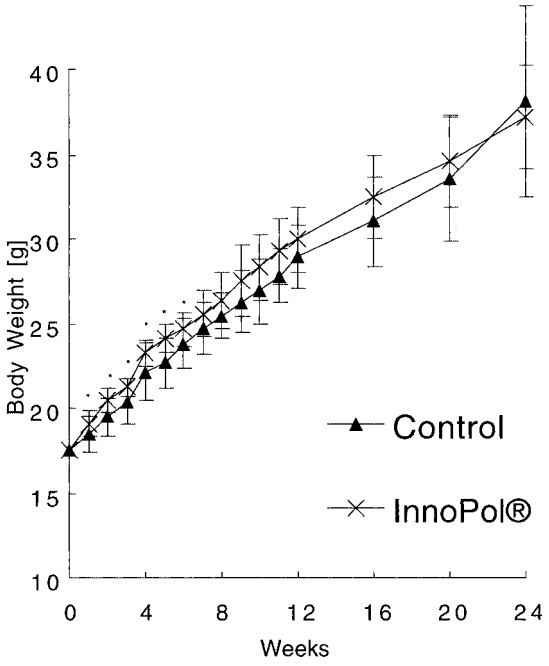

Fig. 1. Body weight changes in mice implanted with poly($_{D,L}$-lactic-co-glycolic acid) [PLGA] 65/35 scaffold, InnoPol® (10 × 3 mm disc). Data are mean ± S.D. *p<0.05, significantly different from the control.

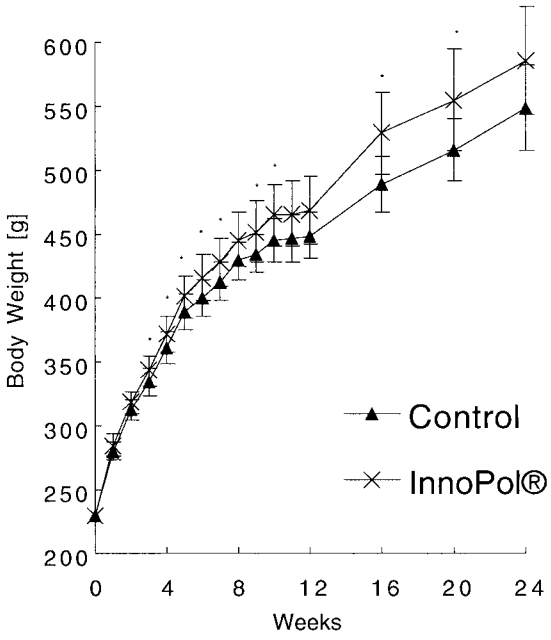

Fig. 2. Body weight changes in rats implanted with poly($_{D,L}$-lactic-co-glycolic acid) [PLGA] 65/35 scaffold, InnoPol® (10 × 1 mm disc). Data are mean ± S.D. *p<0.05, significantly different from the control.

between treatment groups and the control group, Dunnett's test was performed. Treatment values differing from control at the level of $p<0.05$ are indicated with an asterisk. Chi-square test was performed to determine the frequency of lesion.

Results

Mortality and clinical signs

There was no effect of InnoPol® on mortality. No behavioral changes or visible signs of physical impairment indicating systemic or neurological toxicity were observed during the post-operative examinations and at the time of sacrifice in both mice and rats. Gross observation of the implant sites revealed erythema near the operated area in mice (28%), as well as subsequent scar formation and the healing process. Erythema and scar disappeared after 2 month. No other test-item related signs were observed in mice. In the case of rats, there were no treatment-related clinical signs at all.

Body weights

Both treatment group and control group showed consistent weight gain. Mean body weights of male mice implanted with InnoPol® slightly increased from the first to the sixth week after the implantation. However, there was no statistically significant difference between the two groups of mice from the seventh week to the end of study. In contrast, the body weight of male rats implanted with scaffolds was significantly higher at weeks 4, 5, 6, 7, 8, 10, 11, 16, and 20 after implantation (Figs. 1 and 2).

Food consumption

Food consumption of male mice treated with scaffold was significantly increased at weeks 1, 3, 4, 5, 6, 8, 9 and 24 after implantation. This phenomenon is understood to be the requirement of more food consumption during periods of wound healing. The food consumption of male rats implanted with InnoPol® was higher than that of the control group at weeks 2 and 11 (Table 1).

Table 1. Food consumption in male mice and rats implanted with InnoPol®

Group / Week	Mice		Rats	
	Control	Treatment	Control	Treatment
1	3.90 ± 0.48	3.96 ± 0.25*	20.9 ± 0.9	21.1 ± 1.1
2	4.46 ± 0.81	4.05 ± 0.35*	20.4 ± 0.7	21.6 ± 0.9*
3	6.22 ± 0.51	6.68 ± 0.70*	20.9 ± 1.5	21.0 ± 1.0
4	4.45 ± 0.40	6.97 ± 0.72*	20.8 ± 1.1	20.3 ± 1.3
5	4.39 ± 0.37	5.32 ± 0.42*	20.6 ± 0.6	20.5 ± 1.3
6	4.38 ± 0.26	4.49 ± 0.42*	19.8 ± 1.1	20.3 ± 1.0
7	4.33 ± 0.37	4.37 ± 0.47	20.1 ± 1.0	20.1 ± 1.4
8	4.25 ± 0.17	4.35 ± 0.34*	20.2 ± 1.2	20.1 ± 1.4
9	3.67 ± 0.36	3.97 ± 0.32*	18.0 ± 1.3	18.6 ± 2.1
10	4.15 ± 0.60	3.99 ± 0.46	19.1 ± 2.7	21.0 ± 1.5
11	4.29 ± 0.17	4.02 ± 0.37	18.3 ± 1.1	19.9 ± 0.4*
12	4.51 ± 0.27	4.20 ± 0.35	21.5 ± 1.3	21.2 ± 0.8
16	4.36 ± 0.29	4.30 ± 0.51	24.4 ± 0.8	24.8 ± 1.8
20	4.36 ± 0.23	4.22 ± 0.17	22.9 ± 2.8	21.6 ± 1.0
24	4.28 ± 0.02	4.44 ± 0.26*	25.7 ± 3.1	24.2 ± 2.0

Values are presented as mean ± S.D. (g). *, Significantly different from the control ($p<0.05$).

Table 2. Water consumption in male mice and rats implanted with InnoPol®

Group / Week	Mice		Rats	
	Control	Treatment	Control	Treatment
1	5.00 ± 0.97	5.63 ± 0.71*	31.6 ± 2.7	29.7 ± 2.2
2	7.20 ± 1.39	6.59 ± 1.03	31.0 ± 3.4	29.3 ± 3.9
3	9.37 ± 0.95	9.42 ± 0.61	33.8 ± 7.2	31.9 ± 2.9
4	5.41 ± 1.01	7.72 ± 0.66*	32.9 ± 3.4	30.6 ± 3.6
5	4.95 ± 0.87	5.95 ± 0.55*	34.9 ± 2.4	34.9 ± 2.5
6	5.05 ± 0.94	5.45 ± 0.51	31.7 ± 2.5	34.0 ± 1.8
7	4.62 ± 0.46	5.58 ± 0.45*	34.6 ± 3.8	31.2 ± 3.4
8	5.24 ± 0.42	5.50 ± 0.57	34.1 ± 4.5	33.6 ± 1.5
9	5.48 ± 1.12	5.50 ± 0.45	29.1 ± 2.9	27.8 ± 1.8
10	4.99 ± 0.49	5.56 ± 0.71	35.5 ± 5.2	32.1 ± 2.1
11	5.93 ± 1.23	5.59 ± 0.62	35.4 ± 6.1	31.1 ± 2.7
12	4.88 ± 0.72	5.65 ± 0.60*	31.8 ± 6.8	27.8 ± 3.5
16	4.39 ± 0.11	5.54 ± 0.61*	38.8 ± 7.1	32.9 ± 5.1
20	4.65 ± 0.49	5.87 ± 0.52*	36.9 ± 3.4	33.6 ± 5.7
24	4.80 ± 0.47	5.78 ± 0.67*	31.2 ± 1.8	30.5 ± 6.3

Values are presented as mean ± S.D. (g). *, Significantly different from the control ($p<0.05$).

Water consumption

Water consumption of mice treated with InnoPol® was significantly increased at weeks 1, 4, 5, 7, 12, 16, 20 and 24 after implantation and that of male rats implanted with InnoPol® was consistent with the control group (Table 2).

Urinalysis

No significant difference between treatment groups and controls was seen for any of the urinary parameters in both mice and rats (Table 3).

Ophthalmoscopy

Ophthalmologic examinations did not reveal ocular

Table 3. Urinalysis values of mice and rats implanted with InnoPol®

Parameters	Group	Mice		Rats	
		Control (3)[a]	Treatment (5)	Control (5)	Treatment (5)
Specific gravity	1.010	–	–	2	2
	1.015	2	3	1	3
	1.020	1	2	1	–
	1.025	–	–	1	–
pH	6	3	3	2	–
	7	–	2	1	1
	8	–	–	2	4
Leukocytes (Leuko/µl)	0	2	3	–	–
	25	1	2	–	–
	75	–	–	2	4
	500	–	–	3	1
Nitrite	negative	3	5	5	5
	positive	–	–	–	–
Protein (mg/dl)	negative	2	2	1	2
	100	1	3	2	2
	500	–	–	2	1
Glucose (mg/dl)	0	3	5	5	5
Ketone (mmol/L)	negative	3	5	4	5
	5	–	–	1	–
Urobilinogen (mg/dl)	normal	2	4	5	5
	1.0	1	1	–	–
Bilirubin	negative	3	5	5	5
Hemoglobin (Ery/µl)	negative	3	5	4	4
	50	–	–	1	1

[a], Numbers in parentheses are the numbers of animals examined.

lesions in any of the animals (data not shown).

Hematology

In mice, no significant difference between treatment groups and controls was seen for any hematological parameters at 4 weeks. However, there were significant differences in platelet values at 8 weeks, and RBC, Hb, Hct, MCH, and neutrophil counts at 12 weeks between treatment groups and controls. The statistically different values found at 8 weeks and 12 weeks were all within the normal range. There was a statistically significant increase in neutrophil values and a decrease in lymphocyte counts at 24 weeks in mice implanted with scaffold (Table 4).

In rats, there were statistically significant differences in MCV values at 8 weeks, and RBC, Hb, Hct values at 12 weeks, and platelets at 24 weeks between treatment groups and controls. All of these statistically different values were within the normal range (Table 5).

Serum biochemistry

There were no significant differences in the values of serum biochemistry and the blood coagulation test between treatment groups and controls (Table 6).

Gross findings at necropsy and organ weights

Prominent necropsy findings were observed in the implantation sites. Gross observation of the implant sites revealed erythema near the operated area in mice (28%), as well as subsequent scar formation and the healing process, although erythema and scar disappeared after 2 months. There were no other macroscopic observations in any of the male mice and rats of this study.

In mice, absolute weight changes were observed in the lung and heart at 8 weeks. Relative organ weights

Table 4. Hematological values of mice implanted with InnoPol®

Period	4 weeks		8 weeks		12 weeks		24 weeks	
Group	Control (3)[a]	Treatment (9)	Control (3)	Treatment (10)	Control (3)	Treatment (9)	Control (3)	Treatment (9)
WBC (10^3/mm^3)	2.5 ± 1.2	3.7 ± 1.6	3.5 ± 0.6	3.6 ± 1.3	3.5 ± 0.8	3.5 ± 0.9	4.8 ± 2.0	5.8 ± 1.9
RBC (10^6/mm^3)	9.30 ± 0.05	9.05 ± 0.41	8.96 ± 0.22	8.97 ± 0.75	8.96 ± 0.19	9.65 ± 0.24*	9.4 ± 1.5	10.2 ± 0.3
Hb (g/dl)	10.5 ± 2.2	11.4 ± 0.4	13.1 ± 0.3	13.3 ± 1.1	13.2 ± 0.1	13.5 ± 0.3*	12.8 ± 2.0	13.9 ± 0.5
Hct (%)	40.9 ± 9.1	45.4 ± 1.6	43.5 ± 1.0	43.5 ± 3.9	43.4 ± 1.1	46.3 ± 1.3*	44.8 ± 6.8	48.4 ± 1.6
PLT (10^3/mm^3)	875 ± 130.1	757 ± 59.5	653.7 ± 30.1	803 ± 42.9*	755 ± 11.1	770 ± 42.8	502 ± 75.2	498 ± 112.4
MCV (fl)	49.7 ± 0.6	49.8 ± 0.4	48.3 ± 0.6	48.6 ± 0.5	48.7 ± 0.6	48.0 ± 0.5	47.5 ± 1.0	47.3 ± 0.5
MCH (pg)	12.8 ± 0.3	12.6 ± 0.1	14.6 ± 0.1	14.8 ± 0.3	14.7 ± 0.3	14.0 ± 0.3*	13.6 ± 0.2	13.6 ± 0.2
MCHC (g/dl)	25.8 ± 0.38	25.3 ± 0.3	30.1 ± 0.1	30.6 ± 0.6	30.4 ± 0.6	29.2 ± 0.4	28.6 ± 0.4	28.8 ± 0.5
Neutrophils (%)	22.7 ± 18.5	29.2 ± 14.0	49.3 ± 1.5	44.8 ± 11.4	17.7 ± 3.1	28.9 ± 8.2*	25.8 ± 10.9	44.8 ± 9.5*
Eosinophils (%)	0.7 ± 1.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0
Basophils (%)	0.3 ± 0.6	0.1 ± 0.3	0.0 ± 0.0	0.1 ± 0.3	0.3 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Lymphocytes (%)	69.3 ± 23.1	68.0 ± 13.1	49.3 ± 1.5	52.9 ± 11.5	73.0 ± 4.4	65.3 ± 6.3	70.0 ± 9.7	51.7 ± 8.6*
Monocytes (%)	7.0 ± 4.4	2.7 ± 1.5	1.3 ± 1.5	2.2 ± 1.2	9.0 ± 2.7	5.8 ± 3.4	4.5 ± 2.1	3.5 ± 1.7

Each value represents mean ± S.D. [a], Numbers in parentheses are the numbers of animals examined. *, Significantly different from the control ($p<0.05$).

Table 5. Hematological values of rats implanted with InnoPol®

	8 weeks		12 weeks		24 weeks	
	Control (3)[a]	Treatment (10)	Control (3)	Treatment (10)	Control (3)	Treatment (10)
WBC (10^3/mm^3)	7.6 ± 0.2	8.0 ± 1.9	6.7 ± 0.2	7.6 ± 1.2	6.7 ± 2.4	6.3 ± 2.0
RBC (10^6/mm^3)	8.14 ± 0.66	8.01 ± 0.48	8.01 ± 0.20	8.66 ± 0.37*	7.78 ± 0.31	7.57 ± 0.32
Hb (g/dl)	14.9 ± 0.7	14.8 ± 0.6	14.5 ± 0.4	15.2 ± 0.5*	16.7 ± 0.5	16.1 ± 0.7
Hct (%)	41.8 ± 3.9	43.2 ± 2.6	41.6 ± 1.0	45.1 ± 2.0 *	39.9 ± 1.3	38.7 ± 1.8
PLT (10^3/mm^3)	773 ± 7	725 ± 76	765 ± 93	762 ± 47	836 ± 34	776 ± 67*
MCV (fl)	51 ± 1	54 ± 1*	52 ± 1	52 ± 1	51 ± 2	51 ± 1
MCH (pg)	18.3 ± 0.7	18.5 ± 0.7	18.1 ± 0.4	17.6 ± 0.4	21.5 ± 0.7	21.3 ± 0.4
MCHC (g/dl)	35.7 ± 1.9	34.3 ± 0.9	34.9 ± 0.4	33.8 ± 0.7*	41.9 ± 0.9	41.7 ± 0.5
Neutrophils (%)	34 ± 13	37 ± 11	43 ± 7	30 ± 8*	29 ± 11	36 ± 7
Eosinophils (%)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Basophils (%)	0 ± 0	0 ± 0	0 ± 1	0 ± 0	0 ± 0	0 ± 0
Lymphocytes (%)	63 ± 13	60 ± 11	54 ± 9	67 ± 8	68 ± 10	61 ± 8
Monocytes (%)	3 ± 1	3 ± 2	2 ± 3	3 ± 2	3 ± 2	3 ± 2

Each value represents mean ± S.D. [a], Numbers in parentheses are the numbers of animals examined. *, Significantly different from the control ($p<0.05$).

of liver and right kidney at 4 weeks and spleen at 8 weeks were decreased in mice implanted with PLGA scaffold. There was no significant difference in the organ weights of mice at 12 and 24 weeks. In the rats, there was a significant decrease in the mean absolute weights of the heart at 8 weeks, but no difference in the mean relative weights, and no significant differences in the organ weights of rats were observed at 12 and 24 weeks after implantation (Tables 7, 8, 9 and 10).

Histopathology

There were no significant lesions related to the implantation of PLGA 65/35 scaffold, InnoPol®, in the major organs of mice and rats. In microscopical examinations, except for skin, lesions seen in the control group were also observed in the treatment group. Also, metabolic or progressive symptoms including infectious diseases were not observed.

Histopathological examinations of the skin showed

Table 6. Serum biochemical values of mice and rats implanted with InnoPol®

	Mice		Rat	
	Control (6)[a]	Treatment (10)	Control (10)	Treatment (10)
ALT (IU/l)	29.0 ± 4.6	31.0 ± 3.5	44 ± 7	41 ± 6
AST (IU/l)	50.0 ± 4.6	51.2 ± 2.9	137 ± 38	135 ± 34
ALP (IU/l)	52.0 ± 3.6	56.2 ± 9.2	49 ± 7	50 ± 7
TG (mg/dl)	70.0 ± 19.1	81.0 ± 24.1	63 ± 19	70 ± 22
TC (mg/dl)	93.0 ± 7.2	88.4 ± 5.0	127 ± 25	144 ± 19
Glc (mg/dl)	186.7 ± 15.9	206.6 ± 31.5	125 ± 19	122 ± 20
TP (g/dl)	4.5 ± 0.3	4.4 ± 0.2	6.3 ± 0.2	6.3 ± 0.2
Alb (g/dl)	2.8 ± 0.1	2.8 ± 0.0	3.5 ± 0.1	3.4 ± 0.2
A/G ratio	–	–	1.3 ± 0.1	1.2 ± 0.2
BUN (mg/dl)	19.0 ± 2.6	23.0 ± 3.5	22 ± 3	23 ± 3
CRN (mg/dl)	0.4 ± 0.0	0.4 ± 0.0	0.6 ± 0.1	0.6 ± 0.1
TB (mg/dl)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Na (mmol/L)	155.3 ± 0.6	156.0 ± 2.2	145 ± 1	145 ± 1
P (mg/dl)	8.3 ± 0.5	7.9 ± 0.5	5.8 ± 1.0	6.0 ± 0.7
Ca (mg/dl)	9.0 ± 0.3	8.8 ± 0.1	9.8 ± 0.3	9.7 ± 0.2
K (mmol/l)	4.9 ± 0.8	4.4 ± 0.4	4.6 ± 0.2	4.7 ± 0.2
Cl (mmol/l)	120.7 ± 0.6	118.0 ± 3.5	105 ± 2	105 ± 2
PT(sec)	9.0 ± 0.1	8.8 ± 0.2	16.4 ± 0.6	16.2 ± 0.4
aPTT(sec)	29.2 ± 1.1	28.2 ± 0.8	<20	<20

Each value represents mean ± S.D. [a], Numbers in parentheses are the numbers of animals examined.

Table 7. Absolute organ weights of mice implanted with InnoPol®

Week	4 weeks		8 weeks		12 weeks		24 weeks	
Group	Control (3)[a]	Treatment (9)	Control (3)	Treatment (10)	Control (3)	Treatment (10)	Control (6)	Treatment (10)
Body weight (g)	23.89 ± 1.11	24.27 ± 1.08	25.37 ± 0.36	26.05 ± 1.38	30.37 ± 1.08	30.64 ± 2.29	37.53 ± 4.50	37.18 ± 3.08
Brain							0.442 ± 0.013	0.437 ± 0.022
Thymus (g)	0.063 ± 0.006	0.060 ± 0.007	0.057 ± 0.012	0.053 ± 0.007	0.070 ± 0.004	0.072 ± 0.013	0.056 ± 0.021	0.065 ± 0.022
Lung (g)	0.144 ± 0.023	0.144 ± 0.019	0.141 ± 0.012	0.156 ± 0.008*	0.188 ± 0.008	0.184 ± 0.021	0.161 ± 0.025	0.173 ± 0.021
Spleen (g)	0.055 ± 0.006	0.051 ± 0.006	0.057 ± 0.003	0.052 ± 0.005	0.073 ± 0.020	0.061 ± 0.006	0.080 ± 0.029	0.070 ± 0.011
Heart (g)	0.124 ± 0.014	0.126 ± 0.013	0.119 ± 0.015	0.139 ± 0.011*	0.155 ± 0.014	0.155 ± 0.009	0.142 ± 0.011	0.144 ± 0.009
Liver (g)	1.391 ± 0.131	1.254 ± 0.125	1.152 ± 0.003	1.175 ± 0.092	1.610 ± 0.038	1.594 ± 0.105	1.513 ± 0.081	1.488 ± 0.164
Lt. Kidney (g)	0.161 ± 0.003	0.151 ± 0.012	0.146 ± 0.006	0.159 ± 0.010	0.183 ± 0.012	0.185 ± 0.014	0.175 ± 0.016	0.180 ± 0.010
Rt. Kidney (g)	0.163 ± 0.007	0.155 ± 0.009	0.165 ± 0.011	0.158 ± 0.011	0.189 ± 0.011	0.187 ± 0.014	0.177 ± 0.018	0.187 ± 0.020
Lt. Testis							0.071 ± 0.008	0.072 ± 0.007
Rt. Testis							0.073 ± 0.012	0.073 ± 0.006

Each value represents mean ± S.D. [a], Numbers in parentheses are the numbers of animals examined. *, Significantly different from the control ($p<0.05$).

vigorous progression of foreign body reactions as a result of degradation of scaffold after implantation and local inflammatory reactions such as increase of fibroblasts, lymphocyte infiltration and microangiogenesis. Inflammatory reaction progressively diminished as giant cells decreased, and fibroblasts and collagen fibers tended to replace them. Inflammatory reactions were granulomatous inflammation against foreign body, but there was no acute inflammatory reaction due to infection or other causes.

Mice

A summary of the histological responses of InnoPol® in mice is presented in Table 11. At 4 and 8 weeks, a

Table 8. Relative organ weights of mice implanted with InnoPol®

Week	4 weeks		8 weeks		12 weeks		24 weeks	
Group	Control (3)[a]	Treatment (9)	Control (3)	Treatment (10)	Control (3)	Treatment (10)	Control (6)	Treatment (10)
Brain							1.19 ± 0.12[a]	1.18 ± 0.13
Thymus (%)	0.27 ± 0.03	0.25 ± 0.03	0.23 ± 0.04	0.20 ± 0.03	0.23 ± 0.00	0.23 ± 0.03	0.15 ± 0.06	0.18 ± 0.06
Lung (%)	0.60 ± 0.09	0.59 ± 0.07	0.56 ± 0.04	0.60 ± 0.05	0.62 ± 0.05	0.60 ± 0.09	0.43 ± 0.09	0.47 ± 0.07
Spleen (%)	0.23 ± 0.04	0.21 ± 0.03	0.22 ± 0.01	0.20 ± 0.02*	0.24 ± 0.07	0.20 ± 0.02	0.22 ± 0.09	0.19 ± 0.04
Heart (%)	0.52 ± 0.04	0.52 ± 0.05	0.47 ± 0.06	0.53 ± 0.05	0.51 ± 0.03	0.51 ± 0.03	0.38 ± 0.04	0.39 ± 0.03
Liver (%)	5.82 ± 0.44	5.16 ± 0.33*	4.54 ± 0.07	4.51 ± 0.21	5.30 ± 0.09	5.21 ± 0.25	4.09 ± 0.64	4.01 ± 0.45
Lt. Kidney (%)	0.67 ± 0.02	0.62 ± 0.04	0.58 ± 0.01	0.61 ± 0.03	0.60 ± 0.04	0.60 ± 0.04	0.47 ± 0.06	0.49 ± 0.03
Rt. Kidney (%)	0.68 ± 0.04	0.64 ± 0.03*	0.65 ± 0.04	0.61 ± 0.05	0.62 ± 0.02	0.61 ± 0.06	0.48 ± 0.07	0.51 ± 0.07
Lt. Testis (%)							0.19 ± 0.03	0.20 ± 0.02
Rt. Testis (%)							0.20 ± 0.03	0.20 ± 0.02

Each value represents mean ± S.D. [a], Numbers in parentheses are the numbers of animals examined. *, Significantly different from the control ($p<0.05$).

Table 9. Absolute organ weights of rats implanted with InnoPol®

Week	8 weeks		12 weeks		24 weeks	
Group	Control (3)[a]	Treatment (10)	Control (3)	Treatment (10)	Control (10)	Treatment (10)
Body weight (g)	432 ± 19	447 ± 18	450 ± 22	456 ± 24	549 ± 34	586 ± 42
Thymus (g)	0.520 ± 0.113	0.533 ± 0.090	0.354 ± 0.035	0.370 ± 0.071	0.249 ± 0.079	0.254 ± 0.066
Lung (g)	1.695 ± 0.062	1.811 ± 0.222	1.917 ± 0.516	1.922 ± 0.206	2.065 ± 0.377	2.083 ± 0.268
Spleen (g)	0.724 ± 0.037	0.846 ± 0.103	0.740 ± 0.100	0.789 ± 0.059	0.840 ± 0.075	0.890 ± 0.129
Heart (g)	1.264 ± 0.026	1.383 ± 0.062*	1.403 ± 0.100	1.339 ± 0.056	1.542 ± 0.130	1.652 ± 0.119
Liver (g)	13.648 ± 0.125	13.852 ± 1.292	10.785 ± 0.943	10.718 ± 0.987	12.258 ± 1.419	13.425 ± 1.659
Rt. Adrenal (g)	0.026 ± 0.003	0.027 ± 0.004	0.026 ± 0.005	0.027 ± 0.003	0.028 ± 0.005	0.028 ± 0.003
Rt. Kidney (g)	1.120 ± 0.021	1.164 ± 0.119	1.226 ± 0.159	1.275 ± 0.104	1.380 ± 0.132	1.508 ± 0.157

Each value represents mean ± S.D. [a], Numbers in parentheses are the numbers of animals examined. *, Significantly different from the control ($p<0.05$).

Table 10. Relative organ weights of rats implanted with InnoPol®

Week	8 weeks		12 weeks		24 weeks	
Group	Control (3)[a]	Treatment (10)	Control (3)	Treatment (10)	Control (10)	Treatment (10)
Thymus (%)	0.12 ± 0.02	0.12 ± 0.02	0.08 ± 0.01	0.08 ± 0.02	0.045 ± 0.013	0.043 ± 0.010
Lung (%)	0.39 ± 0.02	0.41 ± 0.04	0.42 ± 0.09	0.42 ± 0.06	0.38 ± 0.06	0.36 ± 0.05
Spleen (%)	0.17 ± 0.01	0.19 ± 0.02	0.17 ± 0.03	0.17 ± 0.01	0.15 ± 0.01	0.15 ± 0.02
Heart (%)	0.29 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	0.29 ± 0.01	0.28 ± 0.02	0.28 ± 0.02
Liver (%)	3.2 ± 0.1	3.1 ± 0.2	2.4 ± 0.1	2.3 ± 0.01	2.2 ± 0.2	2.3 ± 0.2
Rt. Adrenal (% × 100)	0.60 ± 0.07	0.60 ± 0.06	0.58 ± 0.08	0.60 ± 0.05	0.50 ± 0.10	0.48 ± 0.05
Rt. Kidney (%)	0.26 ± 0.01	0.26 ± 0.02	0.27 ± 0.02	0.28 ± 0.02	0.25 ± 0.02	0.26 ± 0.02

Each value represents mean ± S.D. [a], Numbers in parentheses are the numbers of animals examined.

mild tissue reaction was observed toward PLGA scaffold, which was characterized by the infiltration of granulocytes, fibroblasts and polymorphonuclear (PMN) cells. Scaffold contained collapsed matrix structures, and some disintegrating collagenous matrix lamellae. All implants were encapsulated by a thin fibrous capsule, in which the capillaries could be observed. The cellular reaction was mainly restricted to the periphery

Table 11. Tissue reactions to InnoPol® in mouse subcutaneous tissues

	4 weeks		8 weeks		12 weeks		24 weeks	
	Control (3)[a]	Treatment (9)	Control (3)	Treatment (10)	Control (3)	Treatment (10)	Control (6)	Treatment (10)
Margin of scaffold								
Collagen	0	+	0	++	0	0	0	0
Fibroblasts	0	+	0	++	0	0	0	0
Giant cells	0	++	0	+	0	0	0	0
Lymphocytes	0	+	0	++	0	0	0	0
Vascularization	0	+	0	++	0	0	0	0
Remnant polymer	0	++	0	+	0	+/–	0	0
Center of scaffold								
Collagen	0	+/–	0	+	0	++	0	+/–
Fibroblasts	0	0	0	+	0	++	0	+/–
Giant cells	0	+	0	+	0	++	0	+/–
Lymphocytes	0	+/–	0	+	0	++	0	+/–
Vascularization	0	+	0	++	0	+/–	0	+/–
Remnant polymer	0	+++	0	++	0	+	0	+/–

0: normal state, +/–: minimum, +: mild, ++: moderate, +++: severe. [a], Numbers in parentheses are the numbers of animals examined.

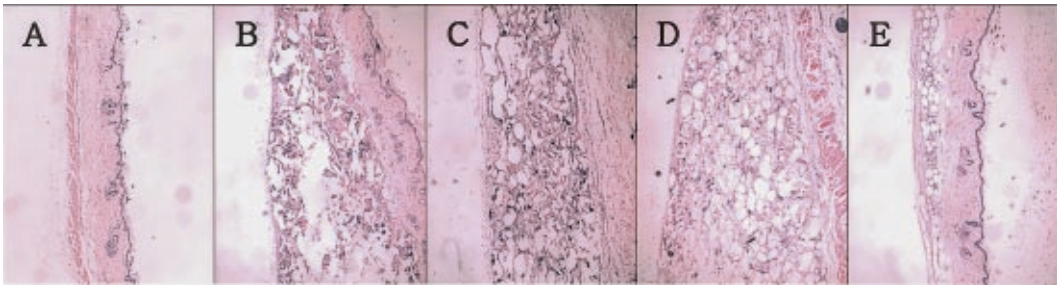

Fig. 3. Histological sections of skin tissues of mice implanted with InnoPol® at different time points stained with H&E. (A) Normal skin at day 0; (B) at 4 weeks, a mild tissue reaction was observed characterized by infiltration of granulocytes, fibroblasts and polymorphoneuclear (PMN) cells; (C) at 8 weeks, all implants were encapsulated by a thin fibrous capsule, in which capillaries could be observed, and giant cells and fibroblasts at the margin of scaffolds had diminished; (D) at 12 week, scaffolds revealed dense regular fibrous structures in between remnants of collagenous lamellae and increased cellular infiltrations were observed in the center of scaffolds; (E) at 24 weeks, cellular infiltrations had further decreased and scaffolds were almost completely degraded. Magnification = 40 ×.

(Fig. 3B and 3C). The tissue response was mainly infiltration of macrophages and fibroblasts, as well as some lymphocytes. PMNs were sporadically present. Giant cells were present at the matrix periphery in varying amounts. At 8 weeks, giant cells and fibroblasts of margin of scaffolds had diminished.

At 12 weeks, InnoPol® scaffolds revealed dense regular fibrous structures in between remnants of collagenous lamellae (Fig. 3D). The margin of scaffolds displayed decreased cellular infiltrations, relative to at 4 weeks, but in contrast, increased cellular infiltrations were observed in the center of scaffolds.

After 24 weeks implantation, cellular infiltrations had further decreased. Scaffolds were almost completely degraded, occasionally leaving several giant cells around small matrix remnants. Loose connective tissue and some fibrous tissues were found at the implant site at the end of 24 weeks (Fig. 3E).

Rats

A summary of the histological responses of InnoPol® in rats is presented in Table 12. Tissue response to-

Table 12. Tissue reactions to InnoPol® in rat subcutaneous tissues

	4 weeks		8 weeks		12 weeks		24 weeks	
	Control (3)[a]	Treatment (9)	Control (3)	Treatment (10)	Control (3)	Treatment (10)	Control (10)	Treatment (10)
Margin of scaffold								
Collagen	0	+	0	+++	0	++	0	+
Fibroblasts	0	++	0	+++	0	++	0	0
Giant cells	0	++	0	++	0	+	0	0
Lymphocytes	0	+	0	+	0	+/–	0	0
Vascularization	0	+	0	++	0	++	0	0
Remnant polymer	0	++	0	+	0	+/–	0	0
Center of scaffold								
Collagen	0	+	0	++	0	+++	0	+/–
Fibroblasts	0	++	0	++	0	+++	0	+/–
Giant cells	0	++	0	+	0	+++	0	+/–
Lymphocytes	0	+/–	0	+	0	+	0	+/–
Vascularization	0	+	0	++	0	++	0	+/–
Remnant polymer	0	++	0	+	0	+	0	0

0: normal state, +/–: minimum, +: mild, ++: moderate, +++: severe. [a], Numbers in parentheses are the numbers of animals examined.

ward InnoPol® in rats was faster than in mice. At 4 weeks, a mild tissue reaction was observed and scaffold contained collapsed matrix structures, and some disintegrating collagenous matrix lamellae. All implants were encapsulated by a thin fibrous capsule, in which capillaries could be observed. The cellular reaction was observed at the periphery and center of scaffolds (Fig. 4A and 4E). The tissue response was mainly infiltrations of macrophages and fibroblasts, lymphocytes and PMNs, as in the case of mice. Giant cells were present at the matrix periphery in varying amounts. At 8 weeks, tissue response at the margin of scaffolds had diminished (Fig. 4B and 4F).

At 12 week, scaffolds had a foamy appearance due to the degradation process. The bundles were surrounded and engulfed by giant cells, resulting in the formation of so-called "islands" of scaffold (Fig. 4C and 4G).

After 24 weeks implantation, cellular infiltrations had further decreased. Scaffolds were almost completely degraded, occasionally leaving several giant cells around small matrix remnants. Loose connective tissues and some fibrous tissues were found at the implant site at the end of 24 weeks (Fig. 4D and 4H), and fibrosis was confirmed with Masson's trichrome staining (Fig. 5).

Discussion

Biodegradable polymers are of interest in medicine because these polymers produce non-toxic degradation products which ultimately circumvent surgical removal of the drug-depleted devices [10]. A number of biodegradable polymers including poly (anhydrides) have been developed for controlled release applications in the last few years. Especially, poly (lactic acid), poly (glycolic acid) and their copolymers are currently being use by the biomedical industry to produce biodegradable sutures, clips and bioactive controlled release devices [1, 4, 5, 13–17, 36, 45, 46]. These polyesters fulfill the requirements of high strength, controlled degradation time, non allergenicity, minimum inflammatory and toxic potential, polymorphism for administration and low cost [6]. So both the use of biomaterials and the number of different applications for biomaterials are increasing. Before a biomaterial can be applied in the clinic, it has to be certified as non-cytotoxic and biocompatible [12].

This study was performed to evaluate subacute systemic toxicity and tissue biocompatibility of a biodegradable three-dimensional polymer scaffold manufactured by InnoTech Medical, Inc., InnoPol®, (Poly ($_{D,L}$-lactide-*co*-glycolide, 65:35): PLGA 65/35) in C57BL/6 mice and Sprague-Dawley rats for 24 weeks. Mice and rats were implanted at subcutaneously with

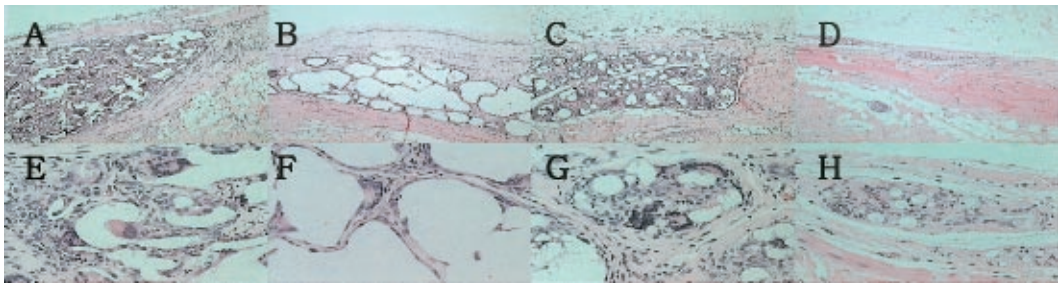

Fig. 4. Histological sections of skin tissues of rats implanted with InnoPol® at different time points stained with H&E. (A) and (E) at 4 weeks, cellular reaction was observed at the periphery and center of the scaffold; (B) and (F) at 8 weeks, tissue response at the margin of scaffolds had diminished; (C) and (G) at 12 weeks, scaffolds had a foamy appearance due to the degradation process and the bundles were surrounded and engulfed by giant cells, resulting in formation of so-called "islands" of scaffold; (D) and (H) at 24 weeks, cellular infiltrations had further decreased and scaffolds were almost completely degraded, occasionally leaving several giant cells around small matrix remnants. Magnification = × 40 for (A), (B), (C) and (D); and × 100 for (E), (F), (G) and (H).

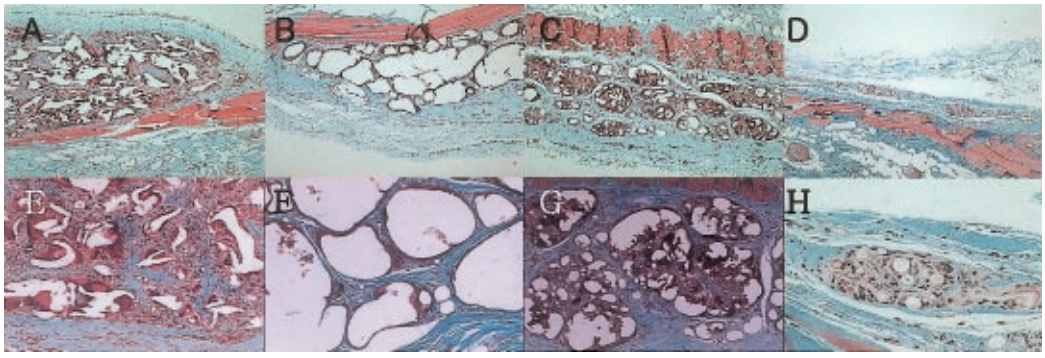

Fig. 5. Histological sections of skin tissues of rats implanted with InnoPol® at different time points stained with Masson's trichrome. (A) and (E) at 4 weeks, (B) and (F) at 8 weeks, (C) and (G) at 12 weeks, and (D) and (H) at 24 weeks. Magnification = × 40 for (A), (B), (C) and (D); and × 100 for (E), (F), (G)and (H).

InnoPol® scaffolds with sizes of 10 × 3 mm and 10 × 1 mm, respectively, and sacrificed at 8, 12, and 24 weeks after implantation. The implanted InnoPol® elicited mild inflammatory reactions throughout the 24 weeks and there was complete degradation of the scaffolds.

Considering that the scaffold itself weighed about 0.023 g, the significant differences in weight by slight increase of mean body weights of mice implanted with InnoPol® in the early period of the study are assumed to decrease. In hematology, the statistically different values in mice and rats of 8 weeks, 12 weeks and 24 weeks were all within the normal range, and there were no significant difference in the values of serum biochemistry and blood coagulation test between treatment groups and controls. Therefore, it was considered that there was no hematological effect due to the implantation of InnoPol®.

Implant sites showed erythema as well as subsequent scar formation and the healing process, near the operated area in mice (28%). This is considered to be a result of inefficient blood and nutrient supply to epidermis and dermis near the implanted area due to the relatively large physical volume of InnoPol® scaffold in respect of body and skin thickness of the mouse. An excessively large scaffold for the mouse's body size and skin thickness was used, and there was a local inflammatory reaction on the skin. Considering these facts, a supplementary study using a smaller scaffold (5 × 2 mm) in order to relieve the local inflammation was conducted to evaluate its effect on dermal regeneration.

The results of this study are not attached to this report, but the local inflammatory reaction, erythema and scab formation were markedly reduced as expected. It also seems that the biodegradation of scaffolds within tissues was performed smoothly. In the study using SD rats, no clinical symptoms that were not shown in mice were observed.

Histopathological examinations of the skin showed vigorous progression of the foreign body reaction as a result of degradation of scaffold after implantation, and the local inflammatory reaction such as increase of fibroblasts, lymphocyte infiltration and microangiogenesis in the early phase. Such changes progressed during the period to 12 weeks after implantation, when scaffold was destroyed either through granulation or breakdown. The inflammatory reaction progressively diminished as giant cells decreased, and fibroblasts and collagen fibers tended to replace them. Inflammatory reactions were granulomatous inflammation against foreign body, and there was no acute inflammatory reaction due to infection or other causes.

In rats, the tendency of the foreign body giant cell reaction to progress vigorously after local inflammatory reactions such as initial increases in numbers of fibroblasts, lymphocyte infiltration and microangiogenesis was identical to tendencies shown in previous trials. In comparison with the mice, biodegradation of scaffold within tissues was delayed, and the degree of local inflammation was relieved. Nonetheless, foreign body giant cell reaction within scaffold, fibroblast infiltration and biocompatibility was remarkably faster in speed the mice. Also, the thickness of the fibroblast layer surrounding the scaffold tended to be higher in comparison with the parts where scaffolds were not implanted.

It is recognized that inflammation around implants is the process of normal host defense mechanisms brought about by the result of surgical implantation, as well as the presence of the implanted materials [22, 35, 41]. In a polymeric implant the inflammatory reaction is dependent on many factors including the extent of injury or defect, size, shape and degradation rates of the polymers as well as the chemical, physical and mechanical properties of the implant materials [9, 18, 39, 44]. Therefore, tissue responses to implanted devices are generally described in terms of acute, subacute and chronic inflammatory reactions, with responses observed over a defined period of time [7, 31]. The tissues adjacent to the implant are then evaluated microscopically relatives to normal tissues [2, 21]. Biodegradable polymers such as polyphosphazenes [45], polyanhydrides [14, 16, 17, 34, 36, 40], and PLGA [3, 8, 46] and many non-degradable polymers [35, 39] have been shown to produce inflammatory responses.

In the supplementary study, fibroblasts were cultivated in the InnoPol® scaffolds and these were compared with the group that was implanted with scaffold only. The trend of local inflammatory reaction and foreign body giant cell reaction were similar, but the number of fibroblasts within the implanted area was relatively higher. Whether these fibroblasts were formed in the healing process of the inflammatory reaction or multiplied from the originally cultured cells needs confirmation.

Increase in fibroblasts is considered a tissue repair reaction. Since infiltrated foreign body giant cells take part in phagocytosis, numerous vascularizations were observed within the cytoplasm. Since it is thought that collagen cannot be produced by fibroblasts, special staining for collagen and other fibers was performed to confirm this. In implanted animals, skin showed local hyperplasia of subcutaneous adipose tissues. Observation under Masson's trichrome staining showed a slight increase in collagen fibers. In addition, cases of foreign substance or inflammation were not seen.

In conclusion, these results suggest that this PLGA 65/35 scaffold, InnoPol®, which possesses good mechanical properties and tissue compatibility, may not cause systemic toxicity except for local inflammatory reaction at the implantation site. Thus, it may be useful as a medical device for implantation, and the results of the current study consequences provide useful information for assessment studies on biocompatibility and safety by implantation.

Acknowledgments

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