Size-Dependency of DL-Lactide/Glycolide Copolymer Particulates for Intra-Articular Delivery System on Phagocytosis in Rat Synovium

Eijiro Horisawa,[1,3] Katsuaki Kubota,[1] Izumi Tuboi,[1] Keiichi Sato,[1] Hiromitsu Yamamoto,[2] Hirofumi Takeuchi,[2] and Yoshiaki Kawashima[2]

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Purpose. The present study evaluated the size-dependency of DL-lactide/glycolide copolymer (PLGA) particulates for an intra-articular delivery system on phagocytosis in the rat synovium after administering directly into the joint cavity. We also investigated the biocompatibility of PLGA particulate systems administered directly into the joint cavity of the rat.

Methods. Fluoresceinamine bound PLGA (FA-PLGA) nanospheres and microspheres were prepared by the modified emulsion solvent diffusion method. The suspension of these particulate systems was administered into the rat-joint cavity and the biological action of the synovium was evaluated by histological inspection and fluorescence microscopy.

Results. A colloidal suspension of the FA-PLGA nanospheres, with a mean diameter of 265 nm, was phagocytosed in the synovium by the macrophages infiltrated through the synovial tissues. The phagocytosed nanospheres were delivered to the deep underlying tissues. An aqueous suspension of the FA-PLGA microspheres, with a mean diameter of 26.5 μm, was not phagocytosed in the macrophages. The macrophages slightly proliferated in the epithelial lining synovial-cells and the microspheres were covered with a granulation of multinucleated giant cells. The molecular weights of the polymer in these particulate systems were slowly reduced in the synovium. Localized inflammatory responses were almost undetected.

Conclusions. PLGA nanospheres should be more suitable for delivery to inflamed synovial tissue than microspheres due to their ability to penetrate the synovium. PLGA particulate systems with biocompatibility in the joint can provide local-therapy action in joint diseases in a different manner depending on the size of the system.

KEY WORDS: DL-lactide/glycolide copolymer nanospheres; DL-lactide/glycolide copolymer microspheres; emulsion solvent diffusion method; intra-articular injection; phagocytosis; macrophage, biocompatibility.

INTRODUCTION

For the treatment of joint diseases, direct intra-articular injections of colloidal anti-inflammatory, rheumatoid drug suspensions have been used to improve the retention of the drug within the joint cavity (1–3). A suspension of drug-incorporated particulate systems with biodegradable polymers has been previously used to prolong the duration of drugs administered via the intra-articular route (4,5). Drug loaded microcapsules directly injected into the joints had a marked local anti-inflammatory effect, and the phagocytosis of synovial macrophage cells was found to be important in maintaining the health of the joints (2,4). For this reason, many researchers have investigated the phagocytosis of macrophages against various microspheres by *in vitro* methods with a cell culture model (6–8) and *in vivo* clinical methods (8–10). However, in these studies the phagocytic mechanism and the periodical activity of the synovial macrophages were not clearly demonstrated, and the generation of macrophages in the synovial tissue was not identified (11,12). Furthermore, the localization and degradation mechanisms of the microparticles administered to the inflamed synovium remain uncertain.

We previously performed a preliminary investigation of a method for encapsulating a water-soluble corticosteroid into the biodegradable nanospheres, which revealed a significant anti-inflammatory action due to their sustained drug releasing properties when administered into the joints of rabbits (13). The aim of the present study was to evaluate the biological response in the synovial tissues after an administration of DL-lactide/glycolide copolymer (PLGA) nanospheres, to clarify their potential use in the local-delivery of such drugs as steroidal anti-inflammatory drugs. The particle size dependency of the nanospheres on localization in the synovium was elucidated from their delivering ability and biocompatibility by histological observations.

MATERIALS AND METHODS

Preparation of Fluoresceinamine Bound PLGA

PLGA with average molecular weights of 19,900 and a copolymer ratio of D,L-lactide to glycolide = 75:25, were supplied by Wako Pure Chemicals Ind. (Osaka, Japan). Fluoresceinamine isomer I (FA) was obtained from Aldrich Chemical Inc. (Milwaukee, Wisconsin). PLGA (3.07 g) and FA (0.0583 g) were dissolved completely in 30 mL of acetonitrile with 0.0408 g of 1-ethyl-3-(3-Dimethylaminopropyl)-carbodiimide hydrochloride (WSC), and incubated at room temperature for 2 h (Fig. 1). The resultant fluoresceinamine bound DL-lactide/glycolide copolymer (FA-PLGA) was washed with distilled water followed by dichloromethane, and then dried using a rotary evaporator (REN-1S, Iwaki, Osaka, Japan).

Preparation of FA-PLGA Nanospheres

FA-PLGA nanospheres were prepared using the modified emulsion solvent diffusion method (14,15). A schematic procedure is shown in Fig. 2. The weighed FA-PLGAs were dissolved in a mixture of methanol and acetone or dichloromethane for nanosphere or microsphere preparations, respectively. The resultant polymer solution was poured into 50 mL of 2% PVA aqueous solution at a constant rate while stirring at 400 rpm (Heidon 600G, Shinto, Osaka, Japan) to produce the polymer emulsion droplets. During evaporation of the organic solvent under reduced pressure (3 h), the coacervated droplets were transformed in the water medium. After completing the diffusion of solvent from the droplets, the entire

[1] Maruho Co., Ltd., Pharmaceutical Research Laboratories 2763, Takamiya-cho, Hikone, Shiga 522-0201, Japan.
[2] Gifu Pharmaceutical University, Department of Pharmaceutical Engineering, 5-6-1, Mitahora-higashi, Gifu 502-0003, Japan.
[3] To whom correspondence should be addressed. (e-mail horisawa_ahl@mii.maruha.co.jp)

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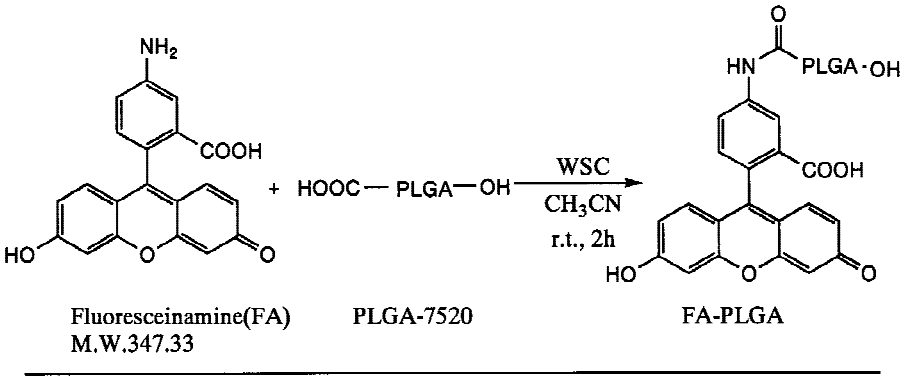

WSC: 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride
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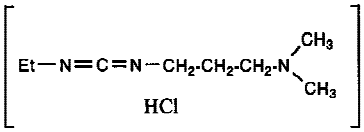

Fig. 1. Schematic procedure for the preparation of FA-PLGA.

dispersed system was centrifuged (43,400 × g for 10 min, Kubota, Osaka, Japan). The sediment was dispersed in distilled water and centrifuged under the same conditions as above. The redispersed particulate system was dried using a freeze dryer (VD-60, TAITEC, Osaka, Japan). The residual organic solvent in the FA-PLGA nanospheres or microspheres was measured by gas chromatography with an FID detector (GC-14A, Shimadzu, Kyoto, Japan, column: Reoflex 400, AW-DMCS, 60-80 Mesh). The sterile FA-PLGA nanospheres and microspheres used for *in vivo* test were prepared at a clean bench (MHE-130A, SANYO Electric, Osaka, Japan).

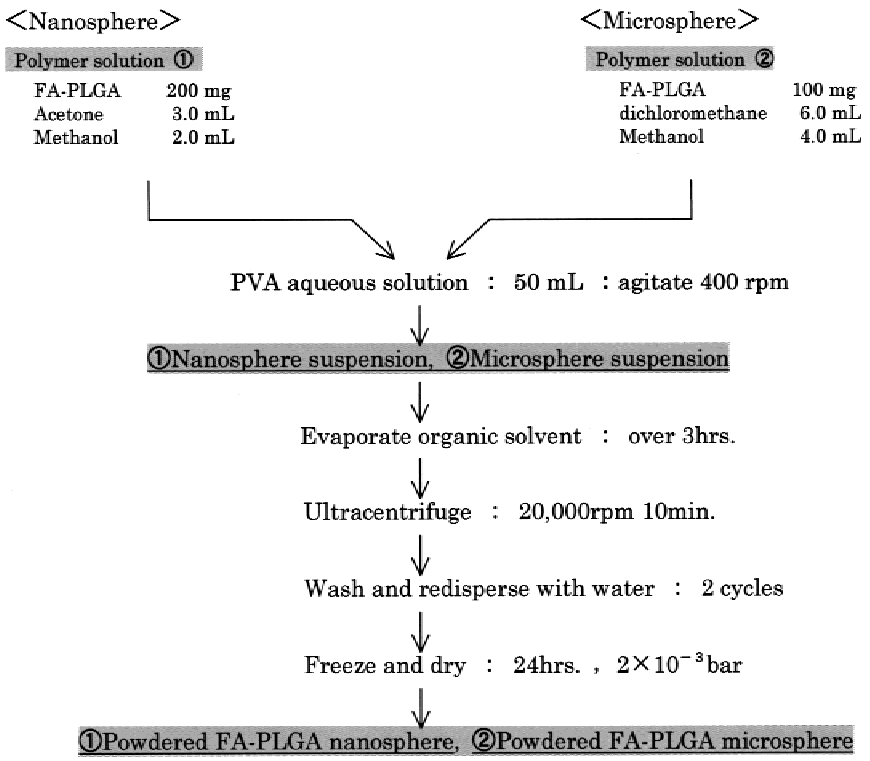

Fig. 2. Schematic procedure for the preparation of the FA-PLGA particulate systems (solvent diffusion method in water, WSD).

Measurement of Particle Size and Size Distribution of FA-PLGA Nanospheres

The mean particle diameter and the size-distribution of the FA-PLGA nanospheres or microspheres in aqueous dispersion were measured by a laser (He-Ne) particle analyzer (LA-700, Horiba, Kyoto, Japan).

In Vivo Study in Rat-Joints

Twelve male wister rats (age: 6 weeks, weight: 250–300 g) were used in the *in vivo* test, and 4 mg of the FA-PLGA nanospheres or microspheres dispersed in 0.1 mL of saline was injected into the left knee joint cavity. Saline (0.1 mL) was injected into the right joint cavity as a control. Within each group, four rats were sacrificed at 1, 3, and 7 days after the injection. The infrapatellar folds containing the synovial membrane and submembraneous adipose tissue were removed, fixed in buffered formalin and embedded in paraffin wax. The required sections were sliced (5 μm), and stained with eosin and haematoxylin, then evaluated by optical and fluorescence microphotography. These sections were also immuno-histochemically stained by the Avidin-Biotin complex method using mouse monoclonal anti-rat macrophages: ED1 and ED2 (Biosource Internationsal). The molecular weights (MWs) and fluorescence intensity of the FA-PLGA nanospheres in the test synovial fluids or tissues from the treated joints homogenized in chloroform were determined by gel permeation chromatography (LC-10A, Shimadzu, Kyoto, Japan, column: 8 × 300 mm, Shodex K-804L, Tokyo, Japan), calibrated with a refractive index detector (RID-10A) and a spectrofluorometric detector (RF-10A), respectively. For calibration to determine the weight-average molecular weight, polystyrenes (Shodex standard series) were used.

RESULTS AND DISCUSSION

Physicochemical Properties of FA-PLGA Particulates

Table I shows the physicochemical properties of the FA-PLGA nanospheres and microspheres. Both freeze-dried FA-PLGA particulate systems exhibited a unimodal particle size-distribution and low standard deviation. The FA-PLGA nanospheres prepared had a mean diameter of 265 nm, ranging from 110 to 670 nm. The FA-PLGA microspheres prepared had a mean diameter of 26.5 μm, ranging from 3.1 μm to 59.9 μm. These values were confirmed with SEM observation and the surfaces of the particles were smooth and rigid, as shown in Fig. 3. Both freeze-dried FA-PLGA particulate systems were successfully redispersed in saline by shaking manually. It was reported previously that the size of the FA-PLGA particulates was mainly determined by the precipitation rate of the polymer and the hardening rate of emulsion droplets in the water phase (14,15). The hardening of emulsion droplets of the FA-PLGA nanospheres became faster than that of microspheres due to the rapid diffusion of the organic solvent, acetone (Fig. 2). The effect of self-emulsifying of dispersed droplets prevented the FA-PLGA nanospheres from agglomerating. The recoveries of the FA-PLGA particulates were high and residual organic solvents in the particles were barely detectable. The molecular weight and the fluorescence intensity of the FA-PLGA particulates after incubating in saline were determined simultaneously by GPC for 5 weeks (Fig. 4). The molecular weight and fluorescence intensity of both FA-PLGA particulates decreased gradually in almost the same manner irrespective of particle size. This finding confirmed that FA was bound to the PLGA particulate systems remained after partial degradation by hydrolysis.

Behavior of Uptaken Phagocyte and Biocompatibility of FA-PLGA Nanospheres in the Synovial Tissue

Figure 5 shows historical photomicrographs of the synovial tissue injected intra-articularly with the FA-PLGA nanospheres. The right joints as a control were normal in appearance. The synovium H.E. stain showed slight proliferation of macrophage cells 1 day after injection (Fig. 5, A1). It was confirmed by ED1 and ED2 that these cells were generated in the synovium and infiltrated through the underlying tissue. The fluorescence microphotograph (Fig. 5, A2) showed the FA-PLGA nanospheres phagocytosed by macrophage cells in the synovial surface membrane. After 3 days, the synovium showed a fairly developed proliferation of macrophage cells (Fig. 5, B1) and the FA-PLGA nanospheres trapped within the cytoplasm of the cells (Fig. 5, B2). These results revealed that the nanospheres administered into the joint cavities were extensively phagocytosed by macrophages existing in the epithelium synovial lining cells and that the macrophages generated through the synovial tissues. In another historical observation by oil red stain, fat particles were not recognized in the synovium. With additional synovial ED stains, it was found

Table I. Physicochemical Properties of the FA-PLGA Nanospheres and Microspheres

Properties	FA-PLGA nanospheres	FA-PLGA microsphere
Mean diameter, D_{50} (nm) ± sd	265 ± 15	26,533 ± 924
D_{90} (nm) ± sd	549 ± 67	47,967 ± 961
Recovery (%)	77	76
Average molecular weight	17,200	17,900
Residual organic solvents[a] (ppm)		
Acetone	ND	ND
Methanol	ND	ND
Dichloromethane	ND	<150
Acetonitrile	ND	ND

[a] The analysis limit of acetone, methanol, and acetonitrile was 50 ppm.

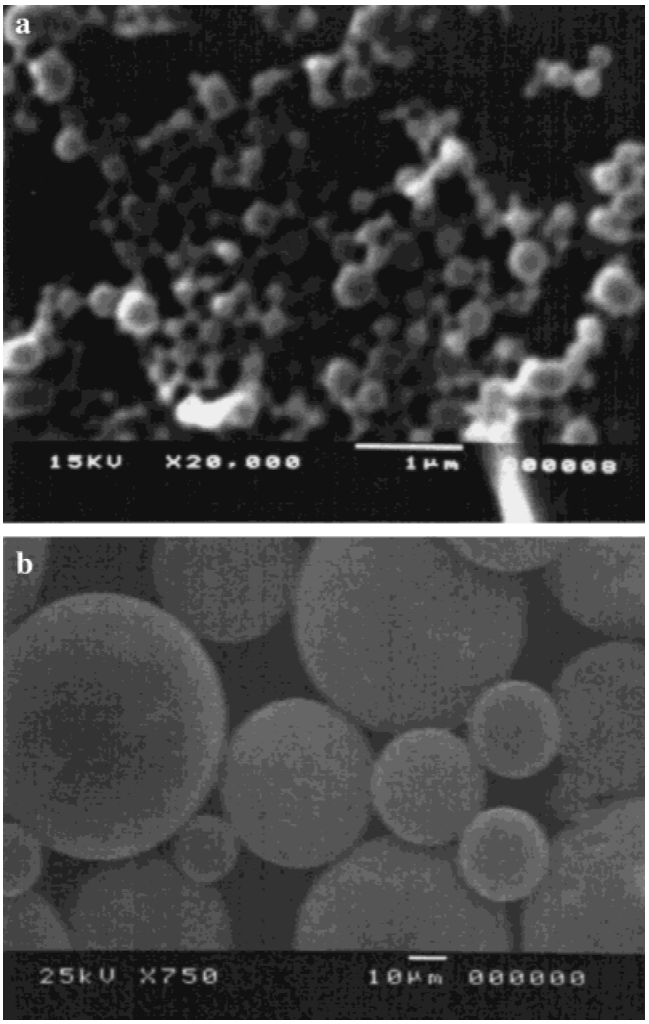

Fig. 3. Scanning electron microphotographs of the FA-PLGA particulate systems: (a):FA-PLGA nanosphere, (b):FA-PLGA microsphere.

that the proliferation of macrophage cells were not induced by the inflammation caused by the injection of FA-PLGA nanospheres but by the elimination of the nanospheres injected in the synovium. After 7 days (Fig. 5, C1), the synovium showed an extensively developed proliferation of the macrophage cells. The fluorescent microphotographs (Fig. 5, C2) indicated that the nanospheres phagocytosed in the macrophages were transferred through the cell-junction and penetrated deeply into the underlying synovial tissues toward the veins.

The changes in fluorescence intensity of the FA-PLGA nanospheres in the synovium proved that the nanospheres transferred deeply from the synovial fluid to the synovial membrane. The MWs of the nanospheres in the synovial fluid and in the synovial membrane continuously decreased in the same manner for 7 days (Fig. 6). Senda *et al.* (16) suggested that both type A and type B synovial cells possess the ability to phagocytose latex particles of 240 nm. They also proposed that the synovial junction is the draining point via the blood circulation. In our study the FA-PLGA nanospheres, having a mean diameter of 265nm (Table I), when administered in the synovial fluid, were readily phagocytosed by the macrophages in the synovial membrane. And the phagocytic uptake by macrophages infiltrating through the underlying tissue continued. Furthermore, the phagocytosed nanospheres were transferred toward the submembraneous adipose tissue through the cell junction. During the 7-day period, fluorescence in the femoral condylar and the tibial plateaus was not detected.

Inflammatory leucocytes were observed slightly after 1 day, but they soon disappeared. The synovial membrane and tissue were virtually normal in appearance, and other localized inflammatory responses were not detected. These findings indicated that the biological action of the FA-PLGA nanospheres administered into the joint cavity was only spontaneous and confirmed the biocompatible properties of the FA-PLGA nanospheres. The slow decrease in the MWs of PLGA in the synovium suggested the degradation of the polymer by macrophages. Such biocompatible and biodegradable properties of the PLGA nanospheres for direct joint administration should be acceptable for the preparation of a targeting particulate system for joint chronic-diseases. In the intra-articularly delivery of such anti-inflammatory drugs, the PLGA nanospheres phagocytosed by the macrophages in the synovium were assumed to achieve the drug-release for the target site in the synovial tissue due to their slow degradation.

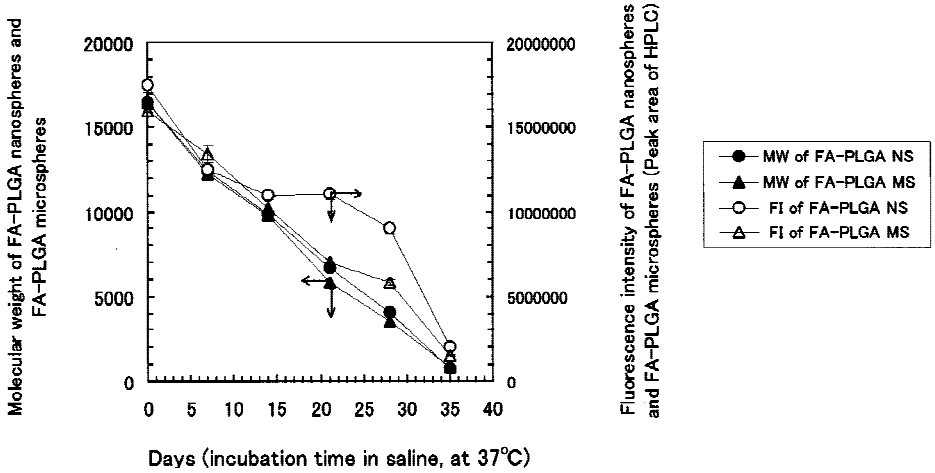

Fig. 4. Molecular weight and fluorescence intensity changes of the FA-PLGA nanospheres and FA-PLGA microspheres incubated in saline.

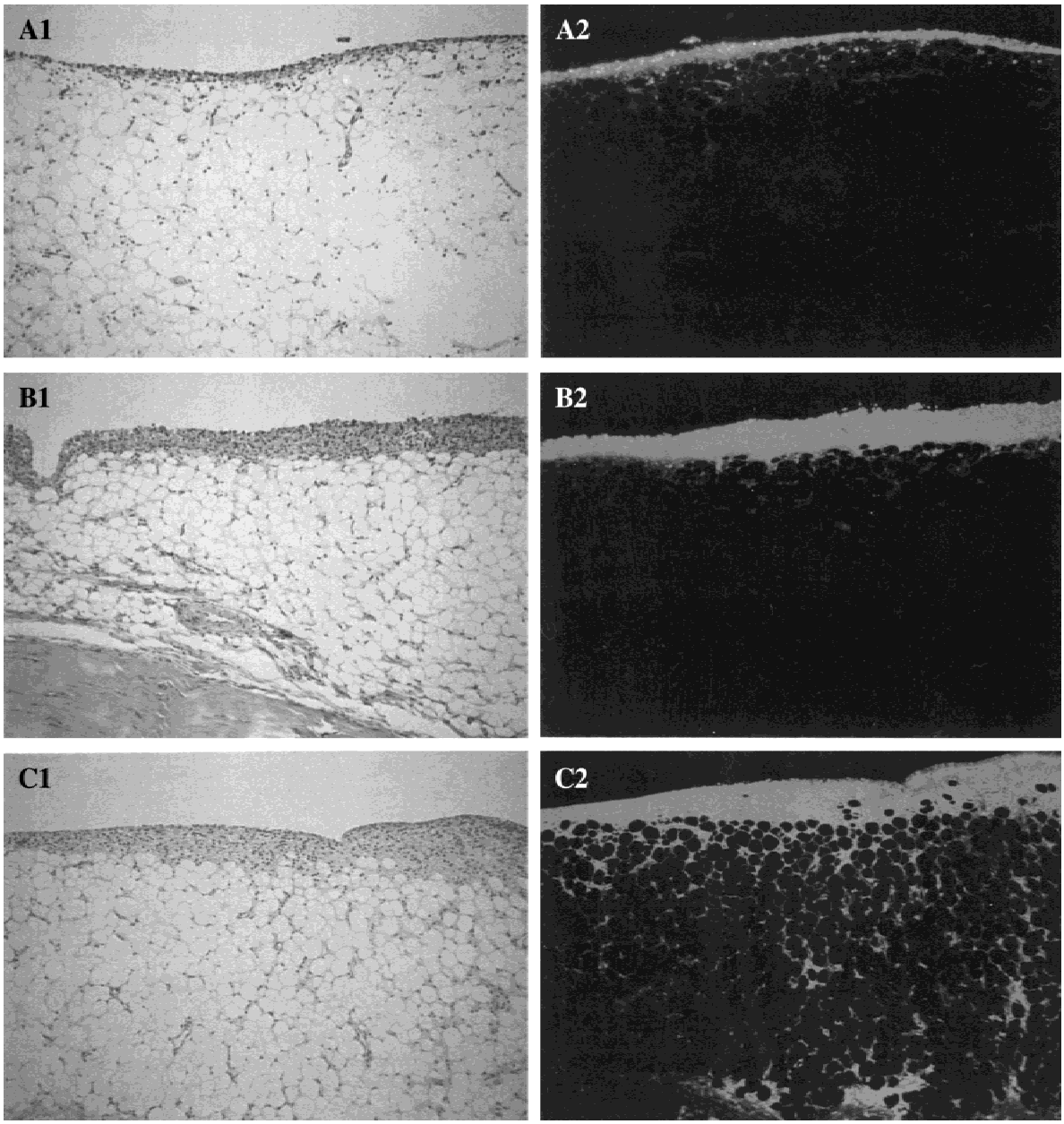

Fig. 5. Photomicrographs of the synovial tissue in the rats injected intra-articularly with the FA-PLGA nanospheres (magnification: × 25). (A1) 1 day after the injection, H.E. stain, (A2) 1 day after the injection, fluorescence, (B1) 3 days H.E. stain, (B2) 3 days fluorescence, (C1) 7 days H.E. stain, (C2) 7 days fluorescence.

Histological Specific Behavior and Biocompatibility of FA-PLGA Microspheres in the Synovial Tissue

Figure 7 shows historical photomicrographs of the synovial tissue injected intra-articularly with the FA-PLGA microspheres. The right joints (control) were normal in appearance. The synovium showed a slight proliferation of macrophage cells at 1 day after injection (Fig. 7, A1), however, the fluorescence of the FA-PLGA microspheres was not detected in the macrophage cells proliferated in the synovial membrane (Fig. 7, A2). The microspheres administered in the joint cavity formed a granulation surrounded by multinucleated giant cells. This finding indicated that there was no phagocytosis by the macrophage cells in the synovial surface membrane and the microspheres were dispersed in the joint cavity or adhered on the surface of articular cartilage and synovium. After 3 days, the granulation was seen over the surface of the synovial membrane (Fig. 7, B1). The fluorescence was observed around the macrophages infiltrated through the synovial tissue (Fig. 7, B2), which was confirmed with immunohistochemical stain : ED1. It has been reported that the synovial macrophages were stimulated immunologicaly by fine microparticles and thereby the microparticles were usually phagocytosed by a natural process (4,16). However, in the present study, the granulation continuously occurred on the synovial surface and was enhanced after 7 days by the infiltrated macrophages (Fig. 7, C1). The fluorescence in the synovium was observed slightly within the surface membrane (Fig. 7, C2). These specific behaviors in Fig. 7 were significantly different from those found in Fig. 5. The findings in

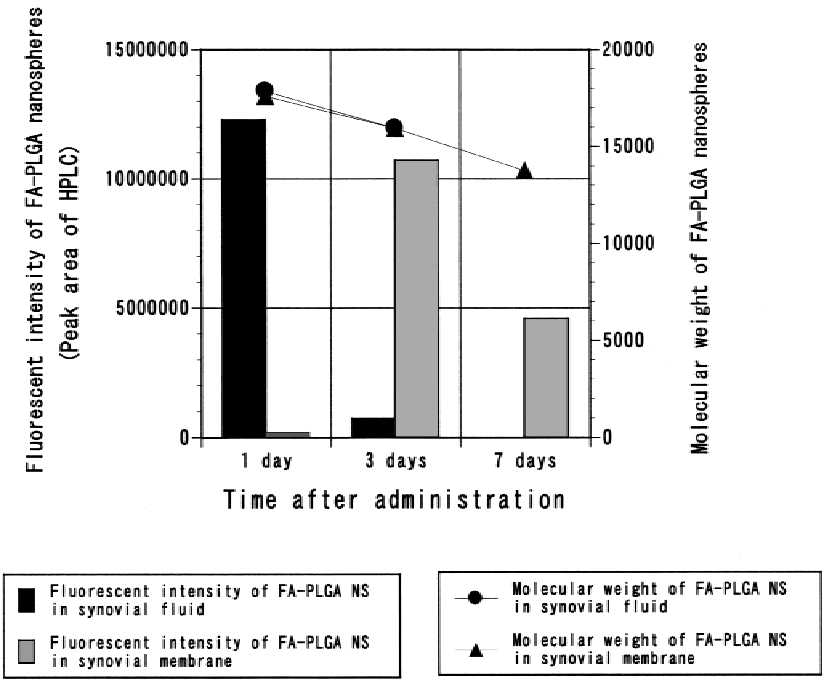

Fig. 6. Molecular weight change and fluorescence intensity of the FA-PLGA nanospheres in the synovial fluid and synovial membrane.

Figs. 5 and 7 indicated that the phagocytosis in the synovium depended strongly on the size of the particulate systems.

The change of fluorescence intensity in Fig. 8 shows that the FA-PLGA microspheres rapidly transferred from the synovial fluid adhering to the synovial surface membrane. This result agreed well with the histological observation in Fig. 7, where the microspheres administered were readily surrounded by the macrophages in the synovial fluid and the resultant granulation adhered to the surface of the synovial membrane. In comparison with FA-PLGA nanospheres, the FA-PLGA microspheres were not transferred through the underlying tissues and the degree of fluorescent intensity was lower because of differences in their surface adhesion in the joint cavity. Howie *et al.* (17) reported that the response of macrophages to particles larger than 5 μm in diameter was approximately the upper limit for biological phagocytosis. They reported that a multinucleated giant-cell response occurred with coarse particles or aggregates of small particles. In this study, fluorescence of the FA-PLGA microspheres was not detected in the synovial membrane during the 7 days. The present microspheres having a mean diameter of 26.5 μm (Table I) were not phagocytosed. These results suggested that phagocytic uptake is caused by the activated cell-viability depending on the particle size.

The inflammatory leucocytes in the synovial tissue were observed slightly only after 1 day and an inflammatory response was not detected. The fibrin or collagen deposition was observed slightly after 1 day, however, the synovial membrane and tissues were normal in appearance. These results in the synovium indicated the safe and biocompatible properties of the FA-PLGA particulate systems. The MWs of the microspheres in the synovial membrane were slightly more reduced than in the synovial fluid at 7 days (Fig. 8). When compared with the FA-PLGA nanospheres, the difference in the degradation was revealed to be due to the difference of the phagocytic behavior. And the tendency toward decreased MWs of the PLGA microspheres suggested the degradation of polymer by the macrophage cells in the synovial surface membrane. These observations suggested that the biocompatible PLGA microspheres are useful for the local delivery of anti-inflammatory drugs on the surface of the synovial membrane.

CONCLUSION

The FA-PLGA microspheres administered into the joint-cavity were surrounded with the macrophages in the synovium, whereas the FA-PLGA nanospheres were rapidly phagocytosed by the macrophages and transferred into the underlying tissue in the synovium. The molecular weights of both FA-PLGA particulates were decreased in the synovium. The histological safety was confirmed for both PLGA particulate systems administered in the synovium. In the intra-articular delivery of anti-inflammatory rheumatoid drugs, the PLGA nanospheres were assumed to be more suitable for delivery to the inflamed synovial tissue than the microspheres due to their penetrating ability in the synovium. The PLGA particulate systems can provide local-therapy action in joint diseases in a different manner depending on the diameter of the system. Based on the present fundamental study, we are preparing PLGA-nanospheres incorporated a model anti-inflammatory drug by the emulsion solvent diffusion method to investigate prolonged anti-inflammatory action and physiological safety in the joints of antigen-induced arthritic rabbit (19).

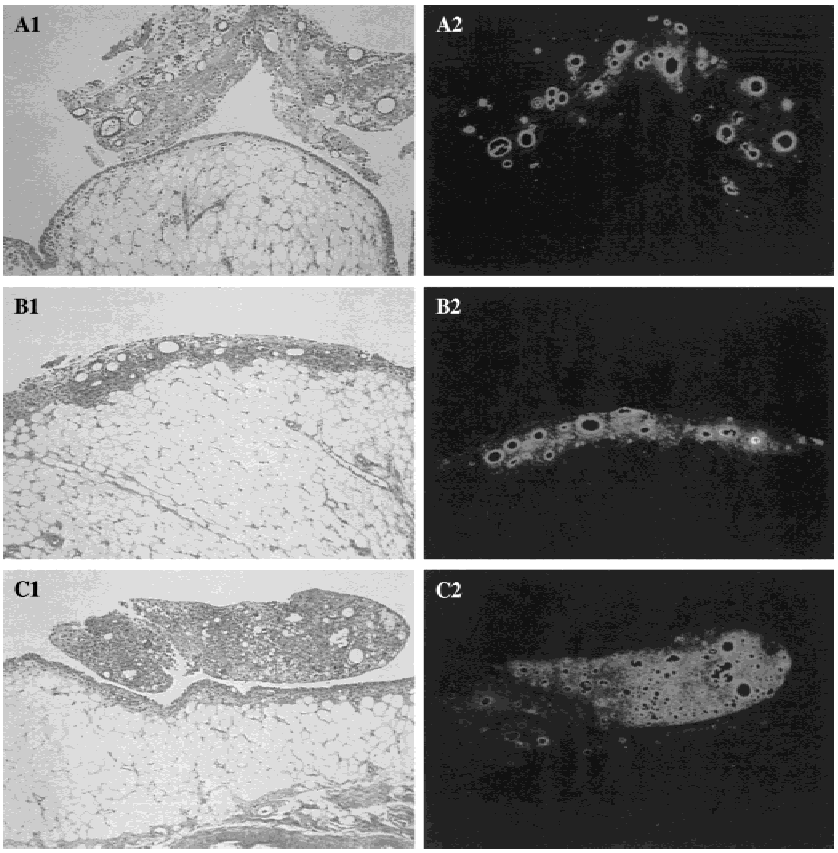

Fig. 7. Photomicrographs of the synovial tissue in the rats injected intra-articularly with the FA-PLGA microspheres (magnification: × 25). (A1) 1 day after the injection, H.E. stain, (A2) 1 day after the injection, fluorescence, (B1) 3 days H.E. stain, (B2) 3 days fluorescence, (C1) 7 days H.E. stain, (C2) 7 days fluorescence.

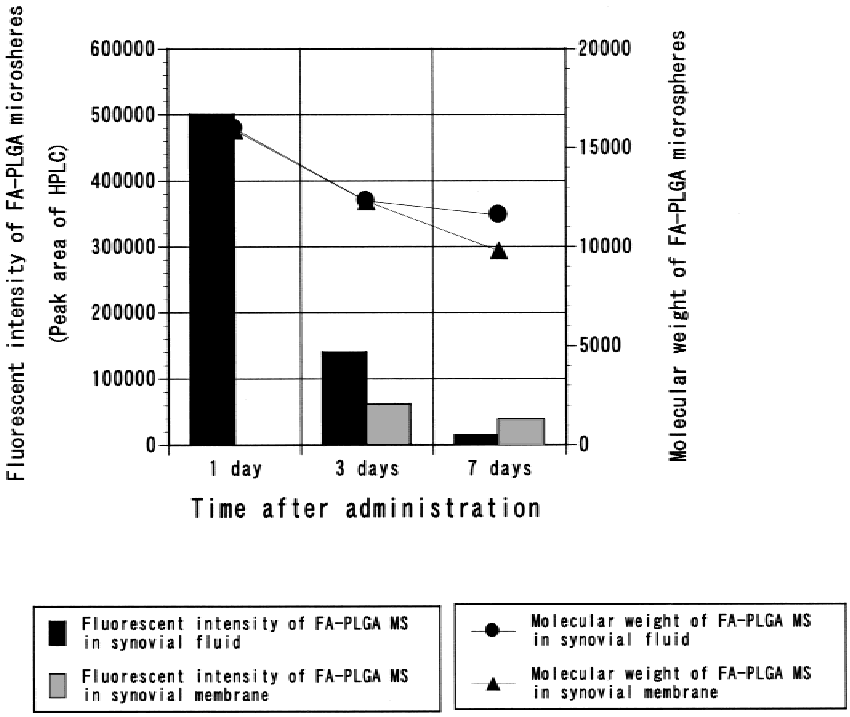

Fig. 8. Molecular weight change and fluorescence intensity of the FA-PLGA microspheres in the synovial fluid and synovial membrane.

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REFERENCES

1. N. C. Phillips, P. Page-Thomas, C. G. Knight, and J. T. Dingle. Liposome incorporated corticosteroids. II. therapeutic activity in experimental arthritis. *Ann. Rheum. Dis.* **38**:553–557 (1979).
2. Y. Mizushima, H. Miyake, K. Fujikawa, N. Ono, and K. Takayama. A highly topically active corticosteroid, *Arzneim.-Forsch/Drug Res. (I)* **30**:274–275 (1980).
3. a) D. J. Burgess, S. S. Davis, and E. Tomlinson. Potential use of albumin microspheres as a drug delivery system. I. preparation and in vitro release of steroids. *Int. J. Pharm.* **39**:129–136. (1987); b) D. J. Burgess and S. S. Davis. Potential use of albumin microspheres as a drug delivery system. II. *in vivo* deposition and release of steroids. **46**:69–76 (1988).
4. J. H. Ratcliffe, I. M. Hunneyball, C. G. Wilson, A. Smith, and S. S. Davis. Albumin microspheres for intra-articular drug delivery: investigation of their retention in normal and arthritic knee joints rabbits. *J. Pharm. Pharmacol.* **28**:290–295 (1987).
5. M. Tuncay, S. Calis, H. S. Kas, M. T. Ercan, H. Erturk, and A. A. Hincal. Dicrofenac sodium incorporated biodegradable microspheres. II: antiinflammatory activity in the knee joints of rabbits, "challenges for drug delivery and pharmaceutical technology, Tokyo": 188 (1998) b) *idem,* Dicrofenac sodium incorporated PLGA (50:50) microspheres : formulation considerations and *in vitro/in vivo* evaluation. *Int. J. Pharm.* **195**:179–188 (2000). c) M. Tuncay, S. Calis, H. S. Kas, M. T. Ercan, I. Peksoy, and A. A. Hincal. *In vitro* and *in vivo* evaluation of dicrofenac sodium loaded albumin microspheres. *J. Microencupsulation* **17**:145–155 (2000).
6. A. S. Shanbhag, J. J. Jacobs, J. Black, J. O. Galante, and T. T. Glant. Macrophage / particle interactions: effect of size, composition and surface area. *J. Bio. Material Res.* **28**:81–90 (1994).
7. C. J. Coester, K. Langer, H. V. Briesen, and J. Kreuter. Gelatin nanoparticles by two step desolvation—a new preparation method, surface modifications and cell uptake. *J. Microencupsulation* **17**:187–193 (2000).
8. M. J. D'Souza and P. DeSouza. Preparation and testing of cyclosporine microsphere and solution formulations in the treatment of polyarthritis in rats. *Drug Dev. Ind. Pharm.* **24**:841–852 (1998).
9. P. Goupille, F. Perin, J.-P. Valat, and A. L. Pape. Macrophage targeting with-^{99m}Tc-labeled J001 for scintigraphy of joint inflammation in ovalbumin induced arthritis in rabbits. *Clin. Exp. Rheum* **15**:269–274 (1997).
10. K. E. Brown, K. Leong, C.-H. Huang, R. Dalal, G. D. Green, H. B. Haimes, P. A. Jimenez, and J. Bathon. Gelatin/chondroitin 6-sulfate microspheres for the delivery of therapeutic proteins to the joint. *Arthritis Rheum.* **41**:2185–2195 (1998).
11. G. Linck and A. Porte. Cytophysiology of the synovial membrane : distinction of two cell types of the intima revealed by their reaction with horseradish peroxidase and iron saccharate in the mouse. *Biol. Cell.* **42**:147–152 (1981).
12. G. Meachim and G. Brooke. The synovial response to intra-articular Co—Cr—Mo particles in guinea pigs. *Biomaterials.* **4**: 153–159 (1983).
13. E. Horisawa, K. Kubota, I. Tuboi, K. Sato, H. Yamamoto, H. Takeuchi, and Y. Kawashima. Size-dependency of DL-lactide/glycolide copolymer particulates for intra-articular delivery system on phagocytosis in rat synovium, "annual meeting of the academy of pharmaceutical science and technology, Tokyo" :168 (2001).
14. Y. Kawashima, H. Yamamoto, H. Takeuchi, T. Hino, and T. Niwa. Properties of a peptide containing DL-lactide/glycolide copolymer nanospheres prepared by novel emulsion solvent diffusion method. *Eur. J. Pharm. Bio.* **45**:41–48 (1998).
15. H. Murakami, M. Kobayashi, H. Takeuchi, and Y. Kawashima. Preparation of poly (DL-lactide-co-glycolide) nanoparticles by modified spontaneous emulsification solvent diffusion method. *Int. J. Pharm.* **187**:143–152 (1999).
16. H. Senda, E. Sakuma, I. Wada, H. J. Wang, H. Maruyama, and N. Matsui. Ultrastructural study of cells at the synovium-cartilage junction: response of synovial cells of the rat knee joint to intra-articularly injected latex particles. *Acta Anat Nippon* **74**:525–535 (1999).
17. D. W. Howie, B. Manthey, S. Hay, and B. V. Roberts. The synovial response to intraarticular injection in rats of polyethylene wear particles. *Clin. Orthop.* **292**:352–357 (1993).
18. B. J. Noble, A. G. Jones, M. A. Davis, C. B. Sledge, R. I. Kramer, and E. Livini. Leakage of radioactive particle systems from a synovial joint studied with a gamma camera. *J. Bone Joint Surg.* **65**:381–389 (1983).
19. E. Horisawa, T. Hirota, S. Kawazoe, J. Yamada, H. Yamamoto, H. Takeuchi, and Y. Kawashima. Prolonged anti-inflammatory action of DL-lactide/glycolide copolymer nanospheres containing betamethasone sodium phosphate for an intra-articular delivery system in antigen-induced arthritic rabbit. *Drug Deliv. Sys.* **16**:334 (2001).