Review Article

Recent development of nanomedicine for the treatment of inflammatory diseases

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Recent advance of nanotechnology enables us to develop drug delivery system using nanocarriers. Here I focused on the treatment of inflammatory diseases using nanotechnology, although the most of the pipelines have been developed for cancer treatment so far. First, I described the basic characteristics of nanocarries including liposomes, polymeric micelles, and nanoparticles. Then I showed the therapeutic activity of betamethasone phosphate and FK506 encapsulated in biocompatible and biodegradable blended nanoparticles of PLGA/PLA homopolymers and PEG-block-PLGA/PLA copolymers (stealth nanosteroid or nano-FK) in experimental arthritis models. Various stealth nanosteroids with a size of 45-115 nm were prepared, and then intravenously administered to rats with adjuvant arthritis (AA rats) and/or mice with Type II collagen induced arthritis (CIA mice). The accumulation of stealth nanoparticles with Cy7 in inflamed joints was determined using an *in vivo* imaging system. The observed strong therapeutic benefit obtained with the stealth nanosteroid/nanoFK in experimental arthritis may have been due to prolonged blood circulation and targeting to the inflamed joint in addition to its sustained release *in situ*.

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Introduction

Nanotechnology carried out on the nanoscale-carriers has been developed for this two decades. The delivery of pharmaceuticals can be improved by drug delivery systems (DDS) using therapeutic colloidal nanocarriers, including polymeric nanoparticles, micelles and liposomes (Fig.1). In addition to being safe, these drug delivery systems must possess high loading capacity, extended circulation time and accumulation in the required pathological sites[1,2]. The initial application is just to form nanoparticles to improve the solubility and enhance the bioavailability of poorly soluble drugs. But recently they are recognized as drug carriers for passive targeting. In certain pathological conditions, the permeability of the tissue vasculatutre increases and particulate nanocarrier less than 200 nm can extravasate and localize in the tissues interstitial space of inflamed tissues and solid tumors in a size-dependent manner. Since these tissues have impaired lymphatic drainage, the carriers concentrate in the lesion and increase the concentration of the drugs. Those passive targeting is also

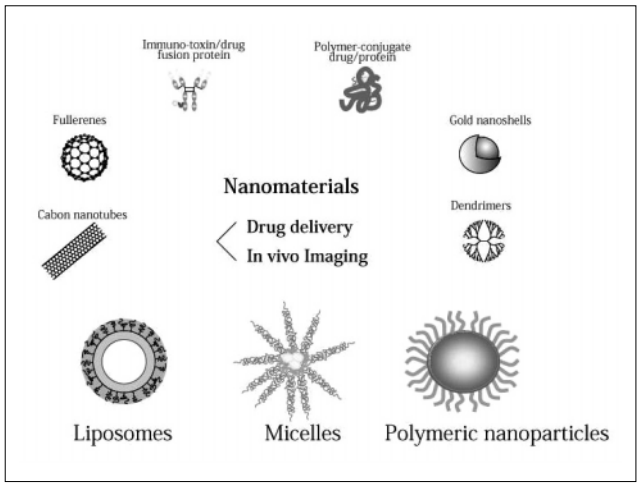


Fig.1 Nanocarriers for drug deliver and *in vivo* imaging

called EPR (Enhanced Permeability and Retention) effect.

Furthermore, drug release might be triggered at the desired site using biodegradable materials or environmental responsive polymers or hydrogels by pH, temperature or magnetic fields etc.

Pharmacological properties of drugs can be improved by using DDS, with particulate nanocarriers, composed of lipids and polymers, by altering the pharmacokinetics and biodistribution of associated drugs[3]. Properties of nanoparticles such as potency in addition to stability, solubility, size and charge are important, although carrier toxicity, metabolism and elimination or biodegradability are problems to be solved.

Nanocarriers

1) Liposome[4]

The attractive property of liposome is its biocompatibility and entrapping hydrophilic pharmaceutical agents in their internal compartment and hydrophobic pharmaceuticals into the membrane, then the incorporated drugs are protected from the inactivation and deliver drug into cells.

Furthermore, size, charge and surface properties can be easily changed and surface-modification with antibody, folate, transferring was carried out[4]. Liposomes are more suitable than polymeric nanoparticles for the encapsulation of hydrophilic small drugs.

Poly (ethylene glycol) (PEG)-grafted liposomes with extended circulation half-lives are in the size range of 70 to 200 nm. MethoxyPEG-2000 grafted to DSPE or DPPE in addition to various amounts of phospholipids and cholesterol. The circulation half-lives is 15 to 24 h and these are the best engineered long-circulating particles. The clinical application of liposomes is well known. The anticancer agent doxorubicin in PEG liposomes (Doxil®/Caelyx®) for the treatment of refractory Kaposi's sarcoma, ovarian cancer, and recurrent breast cancer and liposomal formulation of Amphotericin B (AmBisome®) are employed for the treatment of visceral leishmaniasis. Liposomal photosensitizer verteporin (Visudyne®) that is activated by targeting laser light to blood flowing through the eye causes its site-specific activity in the treatment of wet macular degeneration.

2) Polymeric Micelles[5]

Micelles represent colloidal dispersions (with particle size normally 5-100 nm range) and polymeric micelles are more stable compare to micelles prepared from conventional detergents. Micelles possess an excellent ability to solubilize poorly water-soluble drug within the cores, while polar molecules could be adsorbed on the micelle surface and the substances within intermediated polarity distributed along surfactants molecules in intermediate position. High loading capacity, controlled release profile, and good compatibility between drug and core block, reduction of toxicity and other adverse effects are characteristics. They enhance permeability across barrier due to their small size to pathological areas with compromised leaky vasculature.

Poly (aspartic acid)-b-PEG with doxorubicin and poly (D,L-lactide)b-methoxy-PEG with Paclitaxel® is in a clinical study.

Spherical polymeric micelles with diameters in the size range of 15 to 80 nm structures have been suggested as promising long-circulating carriers of poorly water soluble and amphiphilic drugs.

The stability of micellar systems in vasculature and their extent of interaction with blood and cellular components and the control of drug liberation become a subject of interest.

3) Polymer blended nanoparticles[6,7] (Fig.2)

Polymeric nanoparticles have some advantages over liposomes. It is possible for the drug release profile of polymeric nanoparticles to be modulated, and these nanoparticles are more stable in biological fluids. Additionally, the starting polymers are less expensive than phospholipids, and the manufacturing processes are simple and suitable for industrial scale up, although the use of organic solvents may lead to toxicity issues. Among the polymeric nanoparticles for controlled drug delivery, biodegradable and biocompatible poly (D, L-lactic acid)/poly (D, L-lactic/glycolic acid)/ (PLA/PLGA)-based nanoparticles have been investigated as carriers for therapeutic bioactive molecules, since PLA/PLGA have been studied for many years and are approved by the US Food and Drug Administration for human therapy. The

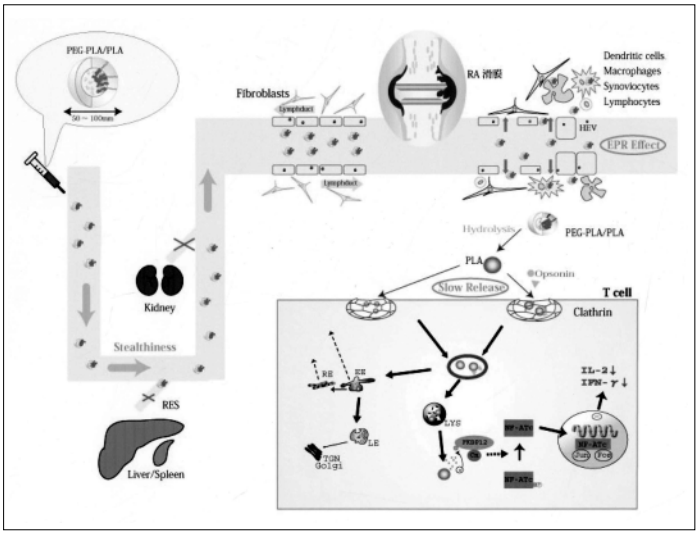


Fig.2 Characteristics of nanoparticles

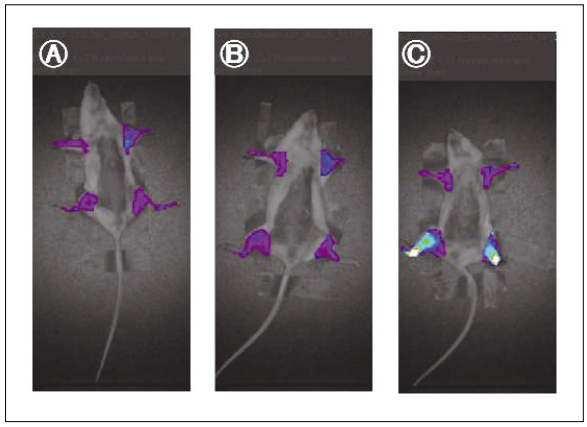


Fig.3 Targeting of stealth nanoparticles to inflamed joints in CIA mice

A; Free Cy7, B; non-stealth nanoparticles, C; stealth nanoparticles

main advantages of PEG nanoparticles compared to other long-circulating systems are their shell stability and their ability to control the release of the encapsulated compound, since PEG-PLA helps to stabilize the inner core, reduce droplet size, and encapsulate drugs. Since a major limitation of polymeric micelles is not only their relatively low loading capacity for water-soluble drugs, but also their inability to engineer PEG content, the mixtures of PLA/PLGA homopolymers and PEG-PLA/PLGA block copolymers allow for the easy adjustment of PEG content of the nanoparticles by simply mixing the appropriate amounts of polymers. Compared to liposomes, polymeric naoparticles (stealth or non-stealth) exhibit the advantage to modulate drug release profile and are more stable after contact with the biological fluids, although the attachment of ligands like antibody is less satisfactory.

Characteristics of nanocarriers

1) Passive Targeting

(1) Targeting to the Lesion

These long-circulating nanoparticles preferentially accumulate in tumors and sites of inflammation with leaky vasculature due to EPR effect (Fig.3). The size of fenestrae in certain inflammatory vessels as well as tumor capillaries can be up to 700 nm. Currently, there is evidence in support of liposome extravasation when the integrity of the endothelial barrier is perturbed by inflammatory processes.

(2) Liver

The smaller the liposomes (usually those of below 100 nm in diameter), the larger is the contribution of hepatocytes in total hepatic uptake. This is probably a reflection of the size of the fenestrations in the hepatic sinusoidal endothelium, which are -100 to 150 nm in diameter Therefore, the size of long-circulating particles, providing that they are rigid structures, should be in the range of 120 to 200 nm in diameter to substantially avoid particle trapping in space of Disse and hepatic parenchyma.

(3) Spleen

The size and the deformability of particles play a critical role in their clearance by the sinusoidal spleens of humans and rats. Particles must be either small or deformable enough to avoid the splenic filtration process at the walls of venous sinuses, then the long-circulating rigid particles of greater than 200 nm may act as splenotropic agents.

(4) LN

Nanoarticles of less than 10 nm can also leave the systemic circulation through the permeable vascular endothelium in lymph nodes. The sinus endothelium of bone marrow is also capable of removing small-sized particles from the systemic circulation.

2) Stealthiness (PEGylation)

The rapid recognition of intravenously injected colloidal carriers, such as liposomes and polymeric nanospheres from the blood by Kupffer cells, Alternatively, PEG with uncharged, hydrophilic and non-immunogenic properties is an attractive material for surface modification of the nanoparticles to reduce opsonization and prevent interactions with the momonuclear phagocytic system (MPS). PLA/PLGA nanoparticles with PEG grafting escape renal exclusion and the MPS; thus, they have enhanced half-lives in plasma. Surface modification of nanoparticles with PEG

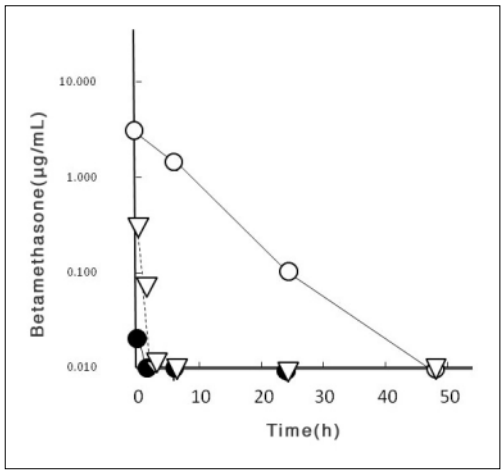


Fig.4 Blood clearance of nanoparticles
○ ; Stealth nanoparticles, ●; Non-stealth nanoparticles, ▽ ; Free Betamethasone

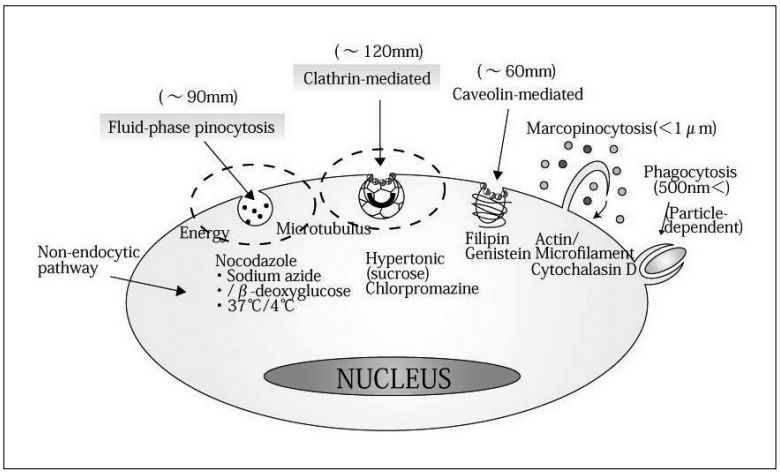


Fig.5 Cellular uptake of nanoparticles
Size and inhibitors

and its derivatives can be performed by adsorption, incorporation during the production of nanoparticles or by covalent attachment to the surface of particles and this stealth capacity of PEG depends on parameters, such as the molecular weight, density, conformation and flexibility of the PEG chains.

The blood clearance of stealth naoparticles are shown in Fig.4.

3) Slow Release (Biodegradibility)

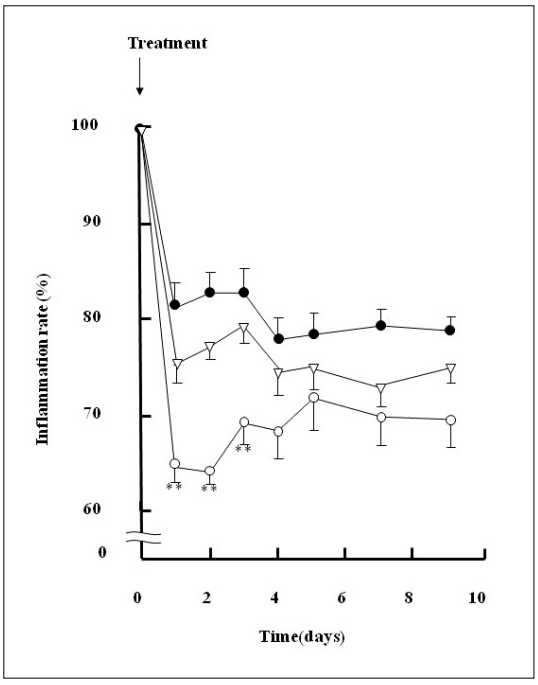
Since the drug might be released in a timely manner, biodegradable polymer, PLGA polymers have been used widely as biomaterials for medical applications over the last 30 years and are regarded as "biocompatible" and "nontoxic". This has been due to the wide variety of materials achievable by varying the molar ratios of the lactic acid and glycolic acid moieties. For example, high molecular weight crystalline PLGA has been used effectively as surgical sutures and bone fixation nails and screws. An example of a successful pharmaceutical product for the controlled delivery of luteinizing hormone in the form of injectable depot is Zoladex®.

4) Cellular uptake (Fig.5)

The propensity of macrophages for endocytosis/phagocytosis of foreign particles has provided an opportunity for the efficient delivery of therapeutic agents to these cells with the aid of colloidal drug delivery systems, following parenteral administration. For other cells, nanoparticles less than 100 nm can enter into cells by pinocytosis, those with diameter below 40 nm can enter the cell nucleus. At sites of inflammation or infection, activated phagocytes may phagocytose extravasated PEG-coated vesicles. The phagocytic process is probably enhanced as a result of the combined effect of an elevated concentration of phospholipase A2 at these sites, and an increased opsonization.

Nanosteroid[8-13)]

Glucocorticoids can be highly effective in treating joint inflammation, but their systemic application is limited because of a high incidence of serious adverse effects, particularly in long-term treatment. As intravenously administered glucocorticoids are distributed throughout the whole body and rapidly cleared, high and frequent dosing is necessary to achieve an effective concentration at inflamed target sites. Moreover, the profound physiological activity of glucocorticoids in many different tissues increases the risk of adverse effects in patients. Thus we need to develop a drug delivery system in particular nanocarriers, with enhanced localization to the target site and sustained drug release. We have engineered nanoparticles with various sustained profiles of drug release and prolonged circulation by blending PLA/PLGA homopolymers and PEG-block-PLA/PLGA copolymers encapsulating betamethasone phosphate. Nanoparticles of different sizes, drug encapsulation/release profiles, and cellular uptake levels were obtained by mixing homopolymers and block copolymers with different compositions/molecular weights at various blend ratios by an oil-in-water solvent diffusion method. Then we examined the therapeutic activity of stealth nanosteroid in experimental arthritis models. Various stealth nanosteroids with a size of 45-115 nm were prepared, and then intravenously administered to rats with adjuvant arthritis (AA rats) and mice with type II collagen induced arthritis (CIA mice). The accumu-



Fgi.6 Anti-inflammatory activity of nanosteroid on AA rats
○ ; Stealth nanosteroid, ●; Non-stealth nanoparticles,
▽ ; Free Betamethasone (40 μg)

lation of stealth nanoparticles with Cy7 in inflamed joints was determined using an *in vivo* imaging system (Fig.4). The stealth nanosteroid composed of PLA (2.6 kD) and PEG (5kd)-PLA(3kd), with a PEG content of 10%, a diameter of 115 nm, exhibited the highest anti-inflammatory activity. In AA rats, a 35% decrease in paw inflammation was obtained in 1 day and maintained for 9 days with a single injection of the stealth nanosteroid (40 μg of BP), while the same does of non-stealth nanosteroid and 3 times higher free BP showed a significantly weaker response (Fig.6). In CIA mice, a single injection of the Type A stealth nanosteroid (3 μg of BP) resulted in complete remission of the inflammatory response after 1 week. Furthermore, in CIA mice, the accumulation of stealth nanoparticles in inflamed joints was shown to parallel the severity of inflammation. The observed strong therapeutic benefit obtained with the stealth nanosteroid in experimental arthritis may have been due to prolonged blood circulation and targeting to the inflamed joint in addition to its sustained release *in situ*.

And these drug carriers could be further targeted by conjugating them with specific ligands. Additionally, these results might apply to other inflammatory or immune diseases for which a steroid is effective.

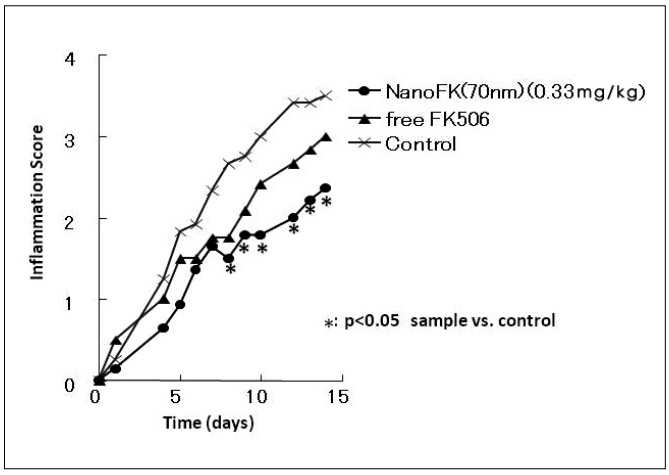


Fig.7 Anti-inflammatoy activity of FK506 containing nanoparticles on CIA mice

Although PEG-liposomes containing prednisolone phosphate for the treatment of experimental arthritis and encephalitis have been reported[14-17], allergic reactions to liposome preparations, even in the PEGylated form, in addition to unstable incorporation, are problematic.

Nano-FK

For the treatment of GVHD and autoimmune diseases, FK506 inaddition to Mycophenolic acid, Cyclosporin A is widely employed.

We have shown the effectiveness of PEG-PLA/PLA nanoparticles containing FK506 for the treatment of CIA and MRL/lpr mice (Fig.7).

The delivery of cyclosporine A to the eye has been supported by the fact that the local bioavailability of this compound improved when it is associated with colloidal carrier such as liposome or chitosan nanoparticles to deliver periocular tisuue. Design nanotechnology able to target different regions, intraocular compartment for the treatment of the ocular uveitis or to the external tissues for the treatment of keratoconjunctivitis sicca.

Summary

As a side effect of naocarrieres, palmer. plantar erythodysesthesia (hand foot syndrome) can appear in patients with pegylated liposomal doxorubicin, because of hypersensitivity by complememt activation. Accumulation in liver, spleen, and bone marrow is another problem of nanocarriers to overcome. Accelerated blood clearance is also recognized, since a single intravenous injection of PEG-grafted long-circulating liposomes into rats or monkeys significantly alters the pharmacokinetic profile

of subsequently injected PEG liposomes. Future considerations must be given toward the immunogenetic and pharmacogenetic differences of nanocarriers.

Nanoparticles across the blood brain barrier for the treatment of Alzheimer and other CNS diseases is another important issue.

Nanocarriers are also promising for synthetic vaccine, delivery of siRNA and oligonucleotide, antibiotic, and small molecule drugs.

References

1) Moghimi SM, Hunter AC, Murray JC: Long-Circulating and Target-Specific Nanoparticles: Theory to Practice. Pharmacol Rev, 53: 283-318, 2001.
2) Couvreur P, Vauthier C: Nanotechnology: Intelligent Design to Treat Complex Disease. Pharmaceut Res, 23: 1417-1450, 2006.
3) Allen TM, Cullis PR: Drug Delivery Systems: Entering the Mainstream. Science, 303: 1818-1822, 2004.
4) Torchilin VP: Recent Advances with Liposomes as Pharmaceutical Carriers. Nature Rev, 4: 145-160, 2005.
5) Torchilin VP: Micellar Nanocarriers: Pharmaceutical Perspectives. Pharmaceut Res, 24: 1-16, 2007.
6) Mundargi REC, Babu VR, Raghavendra CM, Pate P, Aminabhavi TM: Nano/micro technologies for delivering macromolecular therapeutics using poly(D,L-lactide-co-glycolide) and its derivatives. J Control Release, 125: 193-209, 2008.
7) Peracchia MT: Stealth nanoparticles for intravenous administration. S.T.P. Pharam Science, 13: 155-161, 2003.
8) Ishihara T, Izumo N, Higaki M, Shimda E, Hagi T, Mine L, Ogwa Y, Mizushima Y: Role of zinc in formation of PLGA/PLA nanoparticles encapsulating betamethasone phosphate and its release profile. J Control Release, 105: 68-76, 2005.
9) Higaki M, Ishihara T, Izumo N, Takatsu M, Mizushima Y: Treatment of experimental arthritis with poly (D, L-lactic/glycolic acid) nanoparticles encapsulating betamethasone sodium phosphate. Ann Rheum Dis, 64: 1132-1136, 2005.
10) Tamura T, Kita T, Nakagawa T, Endo T, Kim T-S, Ishihara T, Mizushima Y, Higaki M, Ito J: Drug delivery to the cochlea using PLGA nanoparticles. The Laryngoscope, 115: 2000-2005, 2005.
11) Sakai T, Kohno H, Ishihara T, Higaki M, Saito S, Matsushima M, Mizushima Y, Kitahara K: Treatment of experimental autoimmune uveoretinitis with poly (lactic acid) nanoparticles encapsulating betamethasone phosphate. Exp Eye Res, 82: 657-663, 2006.
12) Ishihara T, Takahashi M, Higaki M, Mizushima Y: Efficient encapsulation of a water-soluble corticosteroid in biodegradable nanoparticles. Int J Pharm, 365: 200-205, 2009.
13) Ishihara T, Kubota T, Choi T, Higaki M. Treatment of experimental arthritis with stealth type PLA/PLGA and PEG-PLA/PLGA blended nanoparticles encapsulating betamethasone phosphate. J Pharmacol Exp Ther doi:10.1124/jpet.108.150276
14) Metselaar JM, Wauben MHM, Wagenaar-Hilbers JPA, Boerman OC, Storm G: Complete Remission of Experimental Arthritis by Joint Targeting of Glucocorticoids With Long-Circulating Liposomes. Arth Rheum, 48: 2059-2066, 2003.
15) Schmidt J, Metselaar JM, Wauben MHM, Toyka KV, Storm G, Gold R: Drug targeting by long-circulating liposomal glucocorticosteroids increases therapeutic efficacy in a model of multiple sclerosis. Brain, 126: 1895-1904, 2003.
16) Metselaar JM, van den Berg WB, Holthuysen AEM, Wauben MHM, Storm G, van Lent PLEM: Liposomal targeting of glucocorticoids to synovial lining cells strongly increases thrapeutic benefit in collagen type II arthritis. Ann Rheum Dis, 63: 348-353, 2006.
17) Calvo P, Gouritin B, Villarroya H, Eclancher F, Ginnavola C, Klein C, Andreux JP, Couvreur P: Quantification and localization of PEGylated polycyanoacrylate nanoparticles in brain and spinal cord during experimental allergic encephalomyelitis in the rat. Eur J Neuroscience, 15: 1317-1326, 2002.