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Biodegradation and biocompatibility of PLA and PLGA microspheres

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Abstract

A fundamental understanding of the in vivo biodegradation phenomenon as well as an appreciation of cellular and tissue responses which determine the biocompatibility of biodegradable PLA and PLGA microspheres are important components in the design and development of biodegradable microspheres containing bioactive agents for therapeutic application. This chapter is a critical review of biodegradation, biocompatibility and tissue/material interactions, and selected examples of PLA and PLGA microsphere controlled release systems. Emphasis is placed on polymer and microsphere characteristics which modulate the degradation behaviour and the foreign body reaction to the microspheres. Selected examples presented in the chapter include microspheres incorporating bone morphogenetic protein (BMP) and leuprorelin acetate as well as applications or interactions with the eye, central nervous system, and lymphoid tissue and their relevance to vaccine development. A subsection on nanoparticles and nanospheres is also included. The chapter emphasizes biodegradation and biocompatibility; bioactive agent release characteristics of various systems have not been included except where significant biodegradation and biocompatibility information have been provided. © 1997 Elsevier Science B.V.

Keywords: Inflammatory response; Foreign body response; Macrophages; Foreign body giant cells; Hydrolytic degradation; Therapeutic agent release; In vivo tissue response

Contents

1. Introduction

The design and development of biodegradable microspheres containing bioactive agents for therapeutic application require a fundamental understanding of the in vivo biodegradation phenomena as well as an appreciation of cellular and tissue responses which determine the biocompatibility of the bio-

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degradable microspheres. The objective of this chapter is to provide an overview of the biodegradation phenomena and the biocompatibility of biodegradable microspheres. This chapter is a critical review of the literature which addresses the biodegradation and biocompatibility issues and is focused on and limited to in vivo interactions and responses. Therefore, studies which have focused on in vitro evaluation of the biodegradation and bioactive agent release characteristics of biodegradable microspheres have not been included in this chapter. In part, this review has as its foundation the efforts of the senior author over the past two decades to provide a fundamental understanding of biodegradation and biocompatibility phenomena as they relate to biodegradable polymers in controlled release systems as well as other biomedical polymers utilized in various applications [1–4].

This review is in three parts: Biodegradation, Biocompatibility and tissue/material interactions, and Selected examples of poly(DL-lactic acid), poly-(L-lactic acid) and poly(lactide-co-glycolide) microsphere controlled release systems. Numerous articles in the scientific literature of biodegradable microspheres address the significant issue of drug delivery rates from biodegradable microspheres but do not contain significant information relative to biodegradation or biocompatibility issues. Therefore, the information provided in these articles has not been addressed in this review nor have these articles been included as references as they add little to our fundamental understanding of biodegradation and biocompatibility of biodegradable microspheres. Nonetheless, bioactive agent release rates are important components in the design and development of biodegradable microspheres containing bioactive agents for therapeutic applications [5]. Selected examples of systems containing specific bioactive agents and their respective characteristics and properties are presented in this special issue.

2. Biodegradation

It is generally considered that the mechanism of degradation of aliphatic polyester microspheres is a hydrolytic mechanism [3,5,6]. Investigators have considered the possibility that enzyme catalyzed degradation may occur but these studies are not convincing [7]. The hydrolytic mechanism of degradation is strongly supported by the detailed efforts of Vert and coworkers on the chemistry of the hydrolytic degradation mechanism as well as morphological studies of microspheres in in vitro and in vivo systems by Visscher et al. and Ikada et al. [6–19].

Vert and coworkers have carried out extensive studies on the size dependence of the hydrolytic degradation of devices based on lactic and glycolic acid polymers [10]. Both in vivo and in vitro studies have identified a heterogeneous degradation in large-sized devices [6,9,10]. The heterogeneous hydrolytic degradation in these systems is characterized by a rate of degradation in the core which is greater than that at the surface of the device. Unlike large-sized or thick PLA/GA polymer devices, microspheres less than 300 microns in diameter undergo a homogeneous degradation with the rate of degradation of the core being equivalent to that at the surface [8]. Morphological evaluation of microspheres from in vivo studies support the homogeneous hydrolytic degradation of microspheres composed of lactic and glycolic acid polymers.

Factors which can modulate the hydrolytic degradation behaviour of lactide/glycolide homopolymer and copolymer microspheres are indicated in Table 1. The biodegradation kinetics of microspheres prepared with lactide/glycolide excipients have been determined in rats [20–22]. Rats were injected intramuscularly in the leg with norethistrone or lypressin microspheres prepared with radiolabelled poly(DL-lactide-co-glycolide) copolymers of varying composition. The radiolabel was incorporated into the excipient by using ^{14}C-DL-lactide monomer during its polymerization. Fig. 1 shows the in vivo resorption of radiolabelled poly(DL-lactide-co-glycolide) microspheres. Altering the chemical composition by increasing the glycolide mole ratio in the copolymer increases the rate of biodegradation.

Additives through their acidic or basic nature as well as loading level in the case of therapeutic agents may markedly affect the degradation rate of microspheres. Maulding et al. have reported on the acceleration of microsphere degradation rates by incorporation of a tertiary amino compound, thioridazine [23]. These results suggest that the nucleophilic nitrogen of the thioridazine base participated in the degradation of ester bonds as little effect was seen when

Table 1
Factors affecting the hydrolytic degradation behaviour of biodegradable polyesters

– Water permeability and solubility (hydrophilicity/hydrophobicity)
– Chemical composition
– Mechanism of hydrolysis (noncatalytic, autocatalytic, enzymatic)
– Additives (acidic, basic, monomers, solvents, drugs)
– Morphology (crystalline, amorphous)
– Device dimensions (size, shape, surface to volume ratio)
– Porosity
– Glass transition temperature (glassy, rubbery)
– Molecular weight and molecular weight distribution
– Physico-chemical factors (ion exchange, ionic strength, pH)
– Sterilization
– Site of implantation

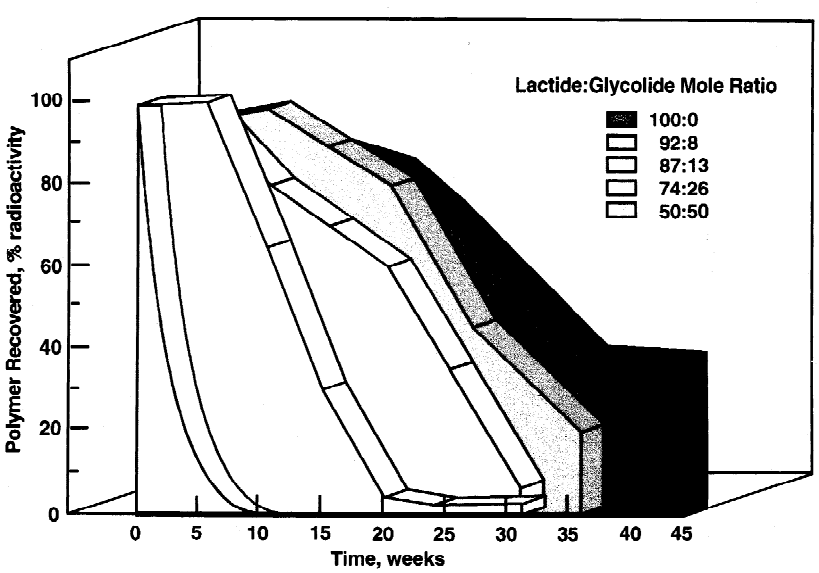

Fig. 1. In vivo resorption rates of radiolabelled poly(DL-lactide-co-glycolide) microspheres injected intramuscularly in rats. The fastest rate of degradation occurs with the 50:50 copolymer and as the glycolic acid content decreases, the rate of degradation decreases. Modified from Figure 3 in Reference [20].

the amino group of thioridazine was protonated and the salt (pamoate) was incorporated in the microspheres. Basic compounds can catalyze ester linkage scission and thus accelerate polymer degradation. On the other hand, appropriate amounts of basic compounds can neutralize carboxyl endgroups and thus decrease the rate of degradation. This potential effect by the acidic/basic nature of the therapeutic agent incorporated in the microspheres must be considered in the design of the microsphere formulation [6]. The crystallinity of the homopolymer or copolymer comprising the microcapsule may play a significant role in modulating the degradation rate. Pistner et al. have carried out long-term in vivo degradation studies of poly(L-lactide) [24]. Utilizing the same batch of poly(L-lactide), these investigators implanted two types of implants subcutaneously in rats and followed the degradation over an extended period of time. The as-polymerized samples were semicrystalline and were microporous with an open structure. The second type of sample that was implanted was an injection-moulded sample which was amorphous and had a tight structure with a solid skin. Their studies indicated that injection moulding caused a decrease in the molecular weight of the implant specimens and that differences in the degradation

behaviour of the amorphous and crystalline samples could be explained by degradation of the amorphous components of the two respective specimens. These authors suggested that the semicrystalline, as-polymerized microporous specimens permitted release of low molecular weight degradation products whereas the degradation of the amorphous, injection-moulded solid specimens did not permit release of low molecular weight degradation products and that autocatalytic degradation was prominent in these specimens. For semicrystalline polyesters, degradation first occurs in the amorphous domains and later in the crystalline regions. During the degradation process, the crystallinity gradually increases resulting in a highly crystalline material which is much more resistant to hydrolysis than the starting polymer [25–28]. The increase in crystallinity has been attributed to an increased mobility of the partially degraded polymer chains due to a larger degree of entanglement which enables a realignment of the polymer chains into a more ordered crystalline state. These results point out the possible effects that post-fabrication treatment, i.e., heat or annealing, as well as shelf time may have on the performance of bioactive agent-loaded microspheres.

Porosity of the microspheres may play a major role in enhancing the rate of biodegradation, especially when the pore dimensions are sufficiently large enough to permit cellular migration into the pores of the microsphere. This effect is demonstrated in the recent effort by Isobe and coworkers where 75:25 poly(DL-lactide-co-glycolide) porous microspheres with bone morphogenetic protein were observed to degrade within three weeks following implantation, whereas non-porous microspheres of 74:26 poly(DL-lactide-co-glycolide) would have been expected to degrade over a time period of 20 weeks [29,22]. Porosity may also enhance the diffusion of oligomers and low molecular weight degradation products whose carboxylic chain ends may facilitate the autocatalytic degradation of the polymer.

In this regard, the molecular weight and molecular weight distribution may play a role in the degradation behaviour. A large molecular weight distribution would indicate relatively large numbers of carboxylic endgroups which can facilitate the autocatalytic degradation of the polymer chains. Large or wide molecular weight distributions thus would be expected to accelerate the rate of degradation whereas a narrow molecular weight distribution would have fewer carboxylic acid endgroups available for autocatalysis.

Given the multiple factors presented in Table 1 which can influence the degradation of microspheres, it is difficult to design microsphere systems for therapeutic agent delivery in which the degradation rates are narrowly specified. Given these multiple factors and the interaction that may occur between therapeutic agent and polymer within microspheres, in vivo studies are necessary to validate the controlled release behaviour and biodegradation rates of any given therapeutic agent/polymer microsphere formulation.

Visscher and colleagues, in an excellent series of studies, have investigated the biodegradation and tissue response to biodegradable injectable microcapsules composed of poly(DL-lactide) and poly(DL-lactide-co-glycolide) [13–16]. In these studies, they have utilized light microscopy (LM, histology), transmission (TEM) and scanning electron microscopy (SEM), carbon radioisotope (^{14}C) and molecular weight evaluation techniques to characterize the biodegradation and tissue responses to the biodegradable microcapsules. It must be noted that in their studies, the microcapsules contained bioactive agents; 1%-lysine-8-vasopressin in 50:50 poly(DL-lactide-co-glycolide), 0.40–1.00% lysine-8-vasopressin in poly(DL-lactide), and 9% ergot alkaloid in 50:50 poly(DL-lactide-co-glycolide). A marked difference in the rate of biodegradation was noted between the poly(DL-lactide) microspheres and the poly(DL-lactide-co-glycolide) microspheres following implantation in rat gastrocnemius muscles. The poly(DL-lactide) microspheres exhibited extensive erosion and breakdown at 360 days and at 480 days only residual particulate was noted in the histological studies. In contrast, the poly(DL-lactide-co-glycolide) microspheres demonstrated extensive erosion and breakdown at 56 days with complete degradation at 63 days. The molecular weight loss studies demonstrated that the 50:50 poly(DL-lactide-co-glycolide) microspheres had a half-life (50% loss of molecular weight) of 15 days.

Fig. 2 demonstrates that there is no significant difference in the rate of polymer degradation as demonstrated by the ^{14}C studies which had lysine-8-vasopressin in the microcapsules as compared to the molecular weight loss studies which evaluated the

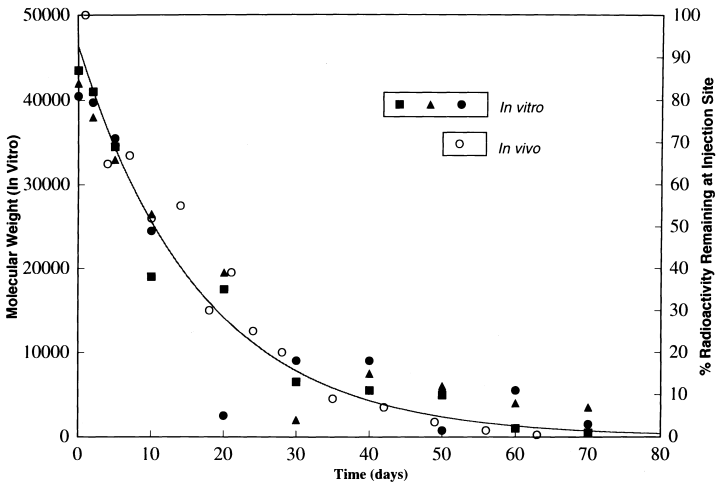

Fig. 2. In vitro-in vivo degradation profile of 50:50 poly(DL-lactide-co-glycolide) microcapsules with lysine-8-vasopressin. The in vivo study was carried out with ^{14}C radiolabelled polymer. Open circles, $X=30$ μm average diameter. In vitro studies were carried out with three different microsphere sizes: closed triangles, $X=30$ μm average diameter, 45–75 μm diameter range; closed circles, $X=79$ μm average diameter, 75–106 μm diameter range; and closed squares, $X=130$ μm average diameter, 106–177 μm diameter range.

effect of microcapsule size on the rate of degradation in microcapsules containing 9% ergot alkaloid. Fig. 2 is a composite taken from Figure 5 of reference 16 and Figure 16 of reference 13. Utilizing the aforementioned microscopic techniques, Visscher and coworkers demonstrated a correlation between the actual breakdown of the microcapsules and their loss of radioactivity. It also appears that the two drugs utilized in the studies had either no effect or the same effect on the rate of in vivo polymer degradation.

Strong evidence for the homogeneous hydrolytic degradation mechanism is given by the comparison of loss of molecular weight of the 50:50 poly(DL-lactide-co-glycolide) in three different size fractions of the ergot-containing microcapsules. The sieve sizes and average particle diameter (μm) were <45–75 ($X=30$); 75–106 ($X=79$); and 106–177 ($X=130$). The authors noted that the largest microcapsules ($X=130$ μm) exhibited a slightly greater tendency to undergo in vivo degradation. However, they concluded that over the microcapsule size ranges examined, minimal differences in the degradation properties of the polymeric matrices of the microcapsules were noted.

The effects of molecular weight and implant site on the in vivo degradation of 75:25 poly(DL-lactide-co-glycolide) microspheres with a diameter range of 53–75 μm have been carried out by Kamei and coworkers [30]. In these studies, two molecular weights of the copolymer were used: 10 000 and 20 000; two implant sites were also used: the subcutaneous tissue and intravenously into the lung. Excellent retrieval and analytical techniques enabled these investigators to demonstrate that the 10 000 molecular weight polymer degraded approximately twice as fast as the 20 000 molecular weight polymer. The 10 000 molecular weight polymer had a half-life of degradation on the order of eight days whereas 18 days was found for the approximate half-life of the 20 000 molecular weight polymer when these molecular weights were used in microspheres and administered subcutaneously in rats. Moreover, it was found that the remaining glycolic acid in the polymer decreased more rapidly than the lactic acid and the lactic acid ratio in the remaining polymer increased with an increase in the weight loss while the L-lactic acid ratio in the lactic acid component was not changed. Degradation in the lung, however, was slightly faster with the 10 000 molecular weight giving a half-life of about four days and the 20 000 molecular weight a half-life of about 14 days. In the lung, the lactic acid ratio in the remaining polymer increased with an increase in the weight loss and the L-lactic acid ratio in the lactic acid was not changed.

The in vivo degradation properties of 75:25 poly(DL-lactide-co-glycolide), average molecular weight of 11 000, have been determined in a microsphere preparation containing thyrotrophin releasing hormone [31]. Analysis of in vivo microspheres demonstrated that the remaining thyrotrophin releasing hormone, microsphere weight and copolymer molecular weight decreased monotonically with an approximate half-life of two weeks for each of these three parameters.

Insight into the final stages of the biodegradation of biodegradable microspheres is found in the elegant experiment by Tabata and Ikada utilizing biodegradable microspheres composed of L-lactic acid, DL-lactic acid, or glycolic acid homopolymers and copolymers of different molecular weights and monomer compositions [17–19]. These investigators studied the phagocytosis of microspheres whose diameter was <2 microns in a mouse peritoneal macrophage cell culture system. Microscopic observation indicated that the phagocytosed microspheres were gradually degraded within the macrophage interior with incubation time. These investigators utilized the release of a fluorescent dye encapsulated in the microspheres to follow the final degradation within the cells. The rate of microsphere degradation in the cells was controlled by changing the molecular weight and the monomer composition of the copolymers comprising the microspheres. The fastest degrading system was comprised of a copolymer with 50 mol % glycolic acid and 50 mol % L-lactic acid. Within seven days, complete degradation within the macrophages was noted for this copolymer. Other copolymers ranging in higher or lower glycolic acid content degraded at a slower rate and microspheres were still identifiable within macrophages at seven days. Tabata and Ikada also examined the influence of protein precoating of the microspheres on the rate of phagocytosis of the microspheres. Precoating with bovine serum albumin and several synthetic polymers reduced the extent of phagocytosis whereas precoating with gelatin, fibronectin and IgG enhanced the rate of phagocytosis consistent with the concept of opsonization.

As microspheres larger than 10 microns in diameter are considered to be too large for phagocytosis, the homogeneous hydrolysis rate leading to degradation and resorption reduces the size of these microspheres to a point where they become susceptible to phagocytosis by macrophages and foreign body giant cells which comprise the foreign body reaction surrounding the larger microspheres. Given the results of the experiments by Tabata and Ikada, it can be anticipated that once the size of microspheres falls into a range where they can be phagocytosed, the degradation from that point will be rapid and most probably much greater than that observed with microspheres >10 microns in diameter.

Macrophages and foreign body giant cells at the interface of the biodegradable microsphere can produce acid and other agents capable of facilitating the biodegradation process. While the buffered physiologic pH is approximately 7, the interfacial pH between the macrophages and foreign body giant cells and the biodegradable polymer surface may be much lower. Phagolysosomal vacuoles within macrophages have been identified as having a pH as low as 3. Thus, cells of the foreign body reaction may produce acidic concentrations which are four orders of magnitude more acidic than extracellular fluid. From a development perspective, it is recommended that in vitro studies be carried out over the pH range of 3 to 7.

3. Biocompatibility and tissue/material interactions

The evaluation of the biocompatibility of implantable delivery systems requires an understanding of the inflammatory and healing responses of implantable materials. Inflammation, wound healing and foreign body responses are generally considered as components of the tissue or cellular host responses to injury [1–4]. The response to injury is initiated by the implantation procedure which in the case of microspheres involves injection of the formulation within a solvent vehicle. Humoral and cellular mechanisms are activated to produce inflammation and healing and the degree to which these mechanisms are perturbed and the extent of the pathophysiologic responses and their resolution are measures of the host reaction to the drug delivery system. Host reactions are considered to be tissue-dependent, organ-dependent and species-dependent.

The sequence of events following implantation of a microsphere drug delivery system is illustrated in Fig. 3 and Table 2. The size, shape, and chemical

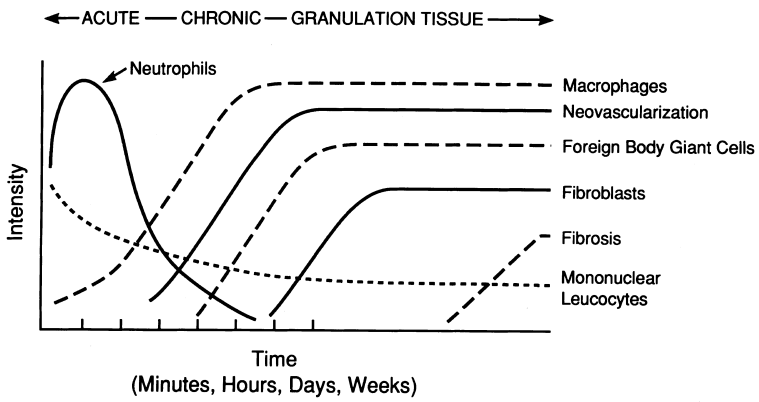

Fig. 3. The temporal variation in the acute inflammatory response, chronic inflammatory response, granulation tissue development, and foreign body reaction to implanted biodegradable microspheres. The acute and chronic inflammatory responses (Phase I) are of short duration, i.e., one to two weeks, regardless of polymer composition of the biodegradable microspheres. The granulation tissue development, foreign body reaction, and fibrosis (fibrous capsule) vary in duration which is dependent upon the rate of polymer biodegradation. Phase III (not shown) follows loss of mass integrity (end of Phase II) and is usually rapid, i.e., weeks. Phase III is characterized by the phagocytosis of microsphere particulate by macrophages and foreign body giant cells.

Table 2
Sequence of local events following implantation: The tissue response continuum

Injury – Injection, Implantation
Acute inflammation – Polymorphonuclear Leukocytes
Chronic inflammation – Monocytes and Lymphocytes
Granulation tissue – Fibroblasts and New Blood Capillaries
Foreign Body Reaction – Macrophages and FBGCs at the Material-Tissue Interface
Fibrosis – Fibrous Capsule

and physical properties of the biomaterial and the physical dimensions and properties of the material, prosthesis, or device may be responsible for variations in the intensity and time duration of the inflammatory and wound healing processes.

Injection of microspheres subcutaneously or intramuscularly results in the implantation of a high surface area/low volume biomaterial into a given tissue volume. Thus, the implant, i.e., volume of microspheres, is distinctly different from the implantation of large-sized devices with comparatively small surface areas. The microsphere implant site is comparable to implant sites seen with open structured foams, porous biomaterials (expanded polytetrafluoroethylene) and fabrics which are knitted, woven or of an open velour structure, i.e., Dacron (Fig. 4 Fig. 5) [2,4].

The sequence of local events following implantation is presented in Table 2 and is generally considered as the tissue response continuum. That is, each individual event leads into the next event from injury to acute inflammation to chronic inflammation to granulation tissue formation to foreign body reaction to fibrous encapsulation. Table 2 also lists the characteristic cell types associated with each event in the tissue response continuum.

In addition to the high surface area/low volume characteristic of implanted microspheres, other characteristic features are noted in the tissue response to this type of implant. As the volume of microspheres within tissue may be considered as an open porous implant, a time lag is present from the onset of acute inflammatory responses at the surface of the microsphere volume to the centre of the microsphere volume. Depending on the packing and the volume of microspheres within an implant site, days to weeks may be required for cellular infiltration from the surface of the microsphere volume to the centre of the microsphere volume. Given the rapid resolution of acute and chronic inflammatory

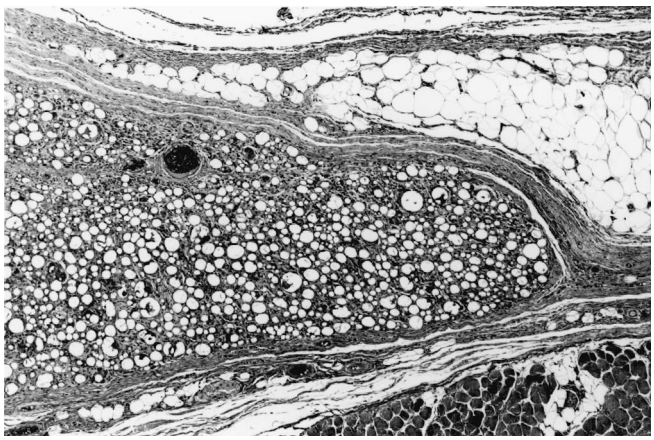

Fig. 4. Histology of Medisorb 65:35 poly(DL-lactide-co-glycolide) microspheres subcutaneously in rats at 75 days. The injection site containing microspheres with the granulation tissue and foreign body reactions are encapsulated in a fibrous capsule which has adjacent skeletal muscle on the bottom and adipose tissue on the top of the figure. An arteriole and venule are present within the injection site (to the left of centre). Original magnification 56X.

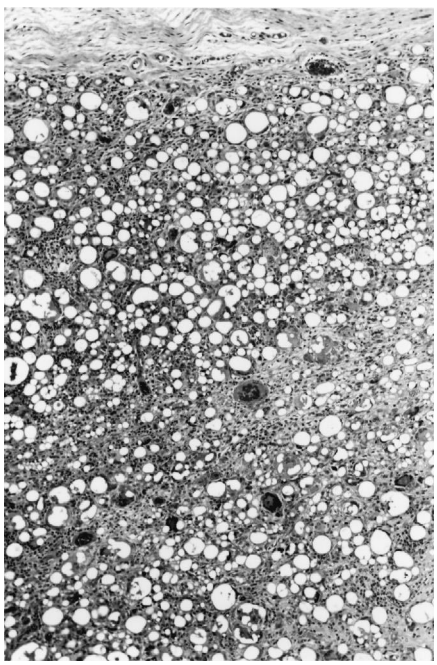

Fig. 5. Histology of Medisorb 65:35 poly(DL-lactide-co-glycolide) microspheres subcutaneously in rats at 90 days. Vascularity within the fibrous capsule is seen at the top of the photomicrograph. In the mid to lower portions of the photomicrograph, a loss of mass integrity of the microcapsules has occurred with an increase in fibroconnective tissue produced by fibroblasts. Original magnification 88X.

responses with biocompatible materials, the cellular components of these responses may resolve quickly and only monocytes, macrophages and fibroblasts may be observed within the central zone of the microsphere implant site (Fig. 6 Fig. 7). The infiltration of inflammatory cells and in particular, monocytes, macrophages and fibroblasts, results in each microsphere having its attendant tissue/material interaction. This is seen as the foreign body reaction with the presence of monocyte-derived macrophages and foreign body giant cells on the surfaces of the microspheres. Foreign body giant cells are formed by the fusion of monocyte-derived macrophages (Fig. 8) [32].

In addition to the individual microsphere tissue response, the volume of microspheres also elicits a response which is generally seen early as a granulation tissue response which leads to fibrous encapsulation of the entire microsphere implant site, i.e., the entire volume of microspheres. This fibrous encapsulation is preceded by a granulation tissue response with fibroblast proliferation and collagen deposition and new capillary formation (neoangiogenesis) (Fig. 5).

Fig. 3 illustrates the temporal variation in the acute inflammatory response, chronic inflammatory response, granulation tissue development, foreign body reaction, and fibrous encapsulation of implanted biomaterials. It should be noted that Fig. 3 illustrates the responses to microspheres which are

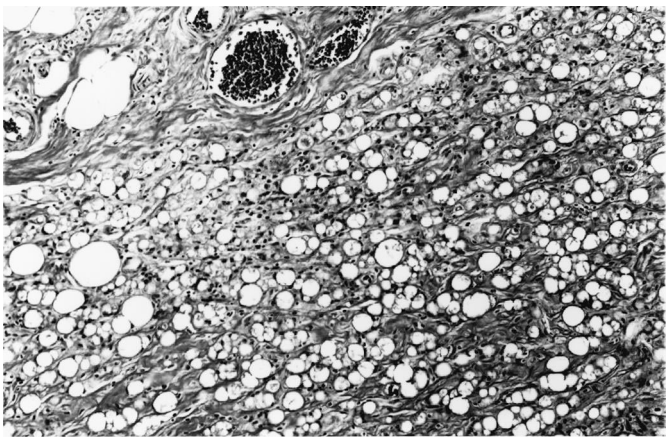

Fig. 6. Histology of Medisorb 65:35 poly(DL-lactide-co-glycolide) microspheres subcutaneously in rats at 90 days. A higher magnification view shows the fibrous capsule with blood capillaries present within fibroconnective tissue. On the right of the photograph, larger channels between microspheres show the presence of increased fibrosis whereas a macrophage predominant reaction with minimal to no fibroblasts is seen below the large blood vessel. Original magnification 140X.

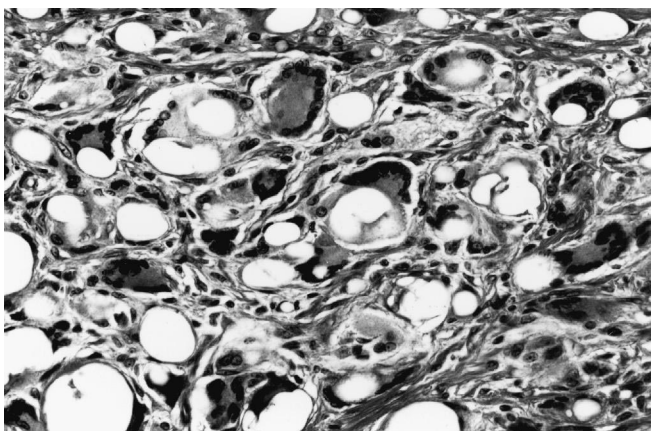

Fig. 7. Histology of Medisorb 65:35 poly(DL-lactide-co-glycolide) microspheres subcutaneously in rats at 90 days. Microspheres of varying sizes and shapes, indicative of biodegradation, are seen as ovoid to irregular open spaces. Multinucleated foreign body giant cells are seen at the tissue/microsphere interfaces. The channels between microcapsules show the presence of fibroblasts identified as having elongated spindle-shaped nuclei and collagen (fibrosis) is present. Original magnification 350X.

larger than 10 microns in diameter. For biocompatible microspheres, the foreign body reaction with interstitial granulation tissue forms within two to three weeks following implantation and is maintained over the time period of degradation to the point where the microspheres are reduced in size to less than 10 microns in diameter [1,33,34]. At this point, phagocytosis of the microspheres by macrophages and foreign body giant cells occurs leading to the final stages of biodegradation and fibrosis. Following complete biodegradation, fibrosis from the implant site fibrous capsule and the fibrosis produced in the granulation tissue response constitutes the implant site. This fibrosis, like all fibrosis in vascularized connective tissue, is metabolically active with collagen being produced and metabolized. With time, the residual fibrosis in the implant site is reduced leaving minimal scar or fibrotic tissue.

The tissue response to injected biodegradable microcapsules may be characterized as occurring in three phases. The first phase (Phase I) occurs within the first two weeks following injection and includes

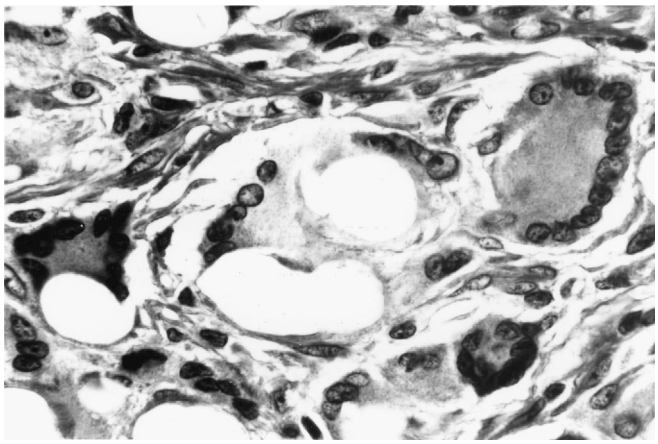

Fig. 8. Histology of Medisorb 65:35 poly(DL-lactide-co-glycolide) microspheres subcutaneously at 90 days. This is a higher magnification view of the implant site seen in Figure 7. Multinucleated foreign body giant cells and macrophages are identified at the interface of the microcapsules. At the top of the photomicrograph, fibroblasts and collagen are identified. The large multinucleated foreign body giant cell to the right is most probably on the top surface of a microsphere which was not sectioned in the preparation of the histology slide. This is a common observation in implant sites. Original magnification 840X.

the initiation, resolution, and organization of the acute and chronic inflammatory responses. Given biocompatible biodegradable microspheres, this response is generally similar regardless of the degradation rate of the biodegradable polymer. A minimal inflammatory reaction with the presence of polymorphonuclear leukocytes, lymphocytes, plasma cells, and monocytes is observed. This early or first-phase response may also contain components of mechanical injury created by injection. Minimal acute myositis along the needle track may be observed with the presence of acute and chronic inflammatory cells. Edema and transient acute myositis are predominantly observed with intramuscular injections as compared to subcutaneous injections. Within days, monocytes predominate in the injection site. Monocytes differentiate into or adopt the phenotype of macrophages which are also known as tissue histiocytes.

The second phase (Phase II) of the tissue response to biodegradable microcapsules is initiated by the predominance of monocytes and macrophages. While the components of the second phase of tissue response are similar, the length of time of their persistence in the injection site is determined by the rate of biodegradation of the microcapsules. Monocytes migrating into the site of injury, i.e., injection site, differentiate into macrophages which in turn may fuse or join together to form foreign body giant cells. Foreign body giant cell formation is surface-dependent and thus is present at the surface or interface of the microcapsules with the tissue. Macrophages may also be observed at the tissue/microcapsule interface. In addition to the local response at the tissue/microcapsule interface, fibrous capsule development is initiated at the onset of the second phase of the tissue response. Fibroblast infiltration with the secretion of collagen, the principal component of the fibrous capsule, and neoangiogenesis, the formation of blood capillaries, is observed surrounding or encapsulating the implant site, i.e., volume of microcapsules, and may also be observed within the interstices or void space between microcapsules.

As previously indicated, the length of time of the second phase of tissue response is dependent upon the rate of biodegradation of the microcapsules. Visscher et al. have shown that 50:50 poly(DL-lactide-co-glycolide) microcapsules have a phase II response of 50 to 60 days whereas the slower degrading poly(DL-lactide) microspheres have a phase II response on the order of 350 to 400+ days [13–16]. As the polymer within the microcapsules degrades, no loss of integrity of the microcapsules may be observed and the phase II tissue response, i.e., the foreign body reaction, remains virtually the

same as observed with light microscopy. Eventually, the biodegradable polymer decreases in molecular weight to the point where the integrity of the microcapsules can no longer be maintained and the microspheres break down into particulate which undergo macrophage phagocytosis and complete degradation.

The third phase (Phase III) of tissue response is characterized by the breakdown into particles of the microspheres. The formation of particles, usually of much smaller size than the microspheres, initiates a tissue response which is predominantly macrophages and the continuing degradation of the polymer occurs quickly under these conditions. The length of time of the phase III response appears to be also dependent upon the degradation rate of the polymer with rapidly degrading polymers exhibiting a phase III response of one to two weeks and slower degrading polymers having a phase III response of several weeks to several months. The fibrous capsule formed during the phase II response is enhanced during the phase III response with fibroblasts and neovascularization filling the volume generated by the loss of microsphere volume.

The variation in inflammatory and foreign body giant cell responses that can be achieved by different routes of administration of biodegradable microspheres as well as different implant sites in tissues or organs is shown in the studies by Spenlehauer and coworkers [8]. Injection of 75:25 poly(DL-lactic-co-glycolic acid) microspheres into the portal veins of rats for embolization within the liver was carried out utilizing cisplatin-free microspheres ranging from 40 to 100 μm in diameter. At 24 h, the microspheres were identified within the small vessels of the liver and neither purulent inflammation nor haemorrhage was observed in the hepatic tissue. At three weeks following embolization, the microspheres were intact, only a few macrophages, lymphocytes and small foreign body giant cells were identified at the surfaces of the microspheres. At four weeks after embolization, an alteration in the shape of the microspheres was noted as well as an increase of the inflammatory response and the number and size of foreign body giant cells. The giant cell formation was more apparent at five weeks together with an increase in the loss of integrity of the microspheres. At six weeks following embolization, only remnants of the 75:25 poly(DL-lactic-co-glycolic acid) microspheres were observed. Total resorption of the microspheres was indicated at eight and 12 weeks following embolization.

Directed intravenous administration of biodegradable microcapsules to target organs can alter the inflammatory and healing responses to the presence of the biodegradable microcapsules. As the injury at the site of the microcapsule within tissue is minimal, only a minimal and very focal inflammatory response with giant cell formation is generated. These investigators also noted that the inflammatory response was identical for microspheres prepared with different polymer compositions. It is noteworthy that they identified no residual fibrosis following the complete resorption of microspheres following venous embolization. In part, this may be due to the fact that collections of microspheres are not present but only single, isolated microspheres are present within the venous network within the liver.

In considering the tissue response to biodegradable microspheres, it is important to consider the biologic activity of the drug or therapeutic agent incorporated into the microspheres, especially if the drug or bioactive agent has demonstrable cytotoxic or anti-inflammatory and wound healing characteristics. Utilizing three controlled release systems different than microspheres, we have demonstrated that gentamycin, naltrexone, and hydrocortisone acetate may alter or modulate the tissue response [34–36].

Gentamycin, while a useful antibiotic, may exhibit cellular toxicity when present in relatively high concentrations. In comparing a gentamycin/silicone rubber controlled release system to silicone rubber, we found that the gentamycin/silicone system exhibited a statistically significant increase in the acute and chronic inflammatory responses (phase I) observed over the first 14 days following implantation [35]. With gentamycin/silicone, the concentrations of polymorphonuclear leukocytes at days four and seven, mononuclear leukocytes at days seven and 14, and alkaline phosphatase activity at day four, were statistically significantly higher when compared to the silicone only implants and controls (no implants) in the in vivo cage implant system. Biocompatibility studies of naltrexone sustained-release formulations utilizing 90:10 poly(L-lactic-co-glycolide) beads demonstrated a cytotoxic effect with the sustained presence of chronic inflammation (monocytes and lymphocytes) at the tissue/bead interface [34]. This focal chronic inflammatory response was not seen with controls consisting of the polymer alone in bead

form. These studies were carried out in subcutaneous implant sites in rats and rabbits and the chronic inflammatory response was observed over the entire 28-day time period of the study for the naltrexone-containing beads. Although little is known regarding the cytotoxicity and inflammatory effects of opioid agonists and antagonists, morphine has been demonstrated to inhibit the chemotaxis of neutrophils and modify the inflammatory response.

In a similar cage implant study, the effect of hydrocortisone acetate-loaded poly(DL-lactide) films on the inflammatory response was evaluated [36]. Over a 21-day in vivo period, hydrocortisone acetate released from the poly(DL-lactide) markedly reduced the accumulation of all types of leukocytes at the site of inflammation. In addition, fibrous capsule formation observed at day 21 was also markedly decreased. Hydrocortisone acetate is a glucocorticoid commonly used as an anti-inflammatory or immunosuppressive agent and at the microscopic level hydrocortisone acetate inhibits edema, fibrin deposition, capillary dilatation, migration of leukocytes, capillary proliferation (neoangiogenesis), fibroblast proliferation, deposition of collagen and scar formation. Thus, therapeutic agents with anti-inflammatory or immunosuppressive properties may markedly affect both phase I and phase II types of reactions.

Studies utilizing cisplatin in 75:25 poly(L-lactic acid/co-glycolic acid) microspheres in rats have indicated an increased inflammatory reaction at the site of the microsphere implantation [37,38]. A comparison of the inflammatory and foreign body reactions to microspheres containing cisplatin suggested that there was a slight increase in these responses compared to cisplatin-free microspheres. This may be another example of the localized response to the drug released from the microspheres. The multiple benefits gained from the use of this form of delivery of chemotherapeutic anti-tumour agents such as cisplatin far outweigh the slight increase in the localized inflammatory reaction.

Prolonged regional nerve blockade studies have been carried out with injectable biodegradable poly-(DL-lactic-co-glycolic acid) microspheres in rats [39]. Although this study did not provide a histological examination of the tissue response, the results from the study are of interest as dexamethasone, an anti-inflammatory agent, was also incorporated within the biodegradable microspheres containing bupivacaine. While the introduction of dexamethasone did not alter the release kinetics of bupivacaine, it was noted that sensory and motor block up to eight–13 fold relative to bupivicaine-polymer microspheres without dexamethasone was achieved. This may be an example of the case where reduction or inhibition of the inflammatory response reduces the localized degradation of a drug and thus increases its bioavailability.

Downregulation of mouse peritoneal macrophage activation has also been identified with prednisolone incorporated in poly(DL-lactic acid) microspheres [40]. These microspheres were in the size range of 1 to 10 μm in diameter and thus were phagocytosed by the mouse peritoneal macrophages. Compared to drug-free microspheres, phagocytosed prednisolone-containing microspheres inhibited or reduced the release of the cytoplasmic enzyme lactate dehydrogenase and the lysosomal enzyme β-glucuronidase. This effect was also noted when the mouse peritoneal macrophages were stimulated with zymosan.

These studies point out two important features of biodegradable microsphere/therapeutic agent systems that must be considered in evaluating the biocompatibility of biodegradable microsphere/therapeutic agent systems. First, the inherent cytotoxicity of the therapeutic agent must be considered as having the potential of altering the inflammatory (phase I) and foreign body reaction (phase II) responses to these types of systems. The histological observation of enhanced or prolonged acute and/or chronic inflammatory responses to these types of systems may be incorrectly attributed to the biodegrable polymer and additives or contaminants which may be present within the polymer. Secondly, these studies point out the importance of carrying out simultaneous control studies utilizing biodegradable microsphere preparations with no therapeutic agents. While it can be anticipated that the majority of biodegradable microsphere/therapeutic agent systems may exhibit no cytotoxicity or no enhanced or prolonged acute and/or chronic inflammation, the potential for therapeutic agents to produce this type of response must be considered in the experimental design and evaluation of such systems.

4. Selected examples of PLA and PLGA microsphere controlled release systems

Over the past decade and, in particular, the past five years, extensive efforts by numerous groups

have been made in the development of poly(DL-lactic acid), poly(L-lactic acid) and poly(lactide-co-glycolide) copolymer microsphere controlled release systems for therapeutic application. As such, these efforts have had as their primary focus the study of release rates of therapeutic agents from the biodegradable microspheres. This section of the review focuses on these publications where significant information relative to biodegradation or biocompatibility has been included in the experimental study. As nanospheres or nanoparticles are a subset of microspheres, nanospheres have been included as a subsection and significant issues dealing with the biodegradation and biocompatibility of nanospheres are included in the review.

The use of biodegradable microspheres and nanospheres for vaccine development is a major area of activity in the utilization of biodegradable microspheres and nanospheres/nanoparticles. However, the vast majority of these efforts, as judged by literature citations, is devoted to the evaluation of mucosal and systemic immunity developed by these systems. Rarely is information pertinent to biodegradation or biocompatibility included in these publications. For this reason, vaccine development has not been included as a subsection but significant issues regarding vaccine development and related to biodegradation and biocompatibility may be found within the lymphoid tissue subsection.

4.1. Bone morphogenetic protein-BMP

Recombinant human bone morphogenetic protein-2 (rhBMP-2) in an aqueous solution of biopolymer with microparticles of poly(DL-lactide-co-glycolide) has been used to accelerate osseous regeneration in critical-size rat calvarial defects [41]. Using Long-Evans rats, the osteoinductive property of this delivery system with rhBMP-2 was investigated over a 21-day time period utilizing radiography, radiomorphometry, histology and histomorphometry. At 21 days, the longest time period studied, significant bone bridging was noted for the rhBMP-2/poly(DL-lactide-co-glycolide) system. The microparticles are still present at 21 days and exhibit the macrophage/foreign body giant cell reaction (Phase II) at their surfaces. In addition to the new bone, bone marrow constituents were also identified in the implant sites.

A similar study has been carried out in large segmental defects in rat femurs utilizing rhBMP-2 in poly(DL-lactide-co-glycolide) [42]. As in the aforementioned study, physical characteristics of the biodegradable polymer were not given. The segmental defect study in rat femurs utilized large (430 μm average diameter) and small (247 μm average diameter) microparticles over the nine-week implantation period. Mechanical property studies were carried out on the rat femurs after nine weeks. Failure torque and torsional stiffness of femurs treated with varying doses of rhBMP-2 with small particle sizes gave the highest mechanical performance. While no histological evaluation was carried out in this study, radiographic measurement of the bone mineral density, bone mineral content, and area of bone formation correlated with the enhanced mechanical properties for the small particles with medium to high doses of rhBMP-2.

Saitoh et al. have investigated the usefulness of polylactic acid as a carrier for bone morphogenetic protein [43]. Polylactic acid particles were mixed with demineralized bone and implanted subcutaneously on the intercostal muscles of rats. Histologic examination revealed cartilage formation at two weeks, new bone formation at four weeks, and extensive bone and marrow formation at 24 weeks. The polylactic acid particles were gradually absorbed and completely disappeared at 24 weeks and were replaced by connective tissue.

Mayer and colleagues have reported on maxillary alveolar cleft repair in dogs using rhBMP-2 with 50:50 poly(lactide-co-glycolide) [44]. The biodegradable polymer particles ranged in size from 125 to 250 μm. Histological evaluation at two months indicated no residual 50:50 poly(lactide-co-glycolide) particles and no cellular responses indicative of the presence of particles, i.e., foreign body giant cells and macrophages, were present. Turk et al., investigated the enhanced healing of large cranial defects utilizing osteoinductive protein with beads of three different formulations of copolymers of lactic and glycolic acid [45]. The different molar ratios of lactic and glycolic acid in the copolymers were stated to result in beads that would resorb in approximately six weeks to six months. No details were given on the exact composition of the beads. Moreover, the beads were coated with poly-2-hydroxymethyl methacrylate. Histological evaluation demonstrated the presence of new bone formation and the presence of macrophages and foreign body giant cells in the foreign body reaction to the beads.

The issue of appropriate and adequate therapeutic doses of rhBMP-2 with biodegradable polymers has been addressed by Hollinger and colleagues [46,47]. These delivery systems use a particulate poly(lactide-co-glycolide) combined with autogenous blood or carboxymethyl cellulose which functions as a carrier system for the rhBMP-2. The rationale for the carrier system is to function as a binder for the particulate poly(lactide-co-glycolide) and aid in localizing the rhBMP-2 at the implantation site.

Poly(α-hydroxy acids) as carriers for bone morphogenetic proteins have been addressed by Hollinger and Leong [48]. In this review, several important issues regarding the biodegradation of poly(α-hydroxy acids) are raised. They note that an ideal delivery system would be one in which the polymer would simultaneously erode with bone regeneration. Unfortunately, poly(α-hydroxy acids) undergo bulk hydrolysis, i.e., bulk erosion, and the particle size does not decrease monotonically with the decrease in molecular weight. A second point raised by these authors is the need for tightly defined temporal biodegradation profiles for the copolymers of lactic and glycolic acid. The presence of crystallinity in the lactic/glycolic acid copolymers may result in phagocytosed particles with their macrophage and foreign body reaction. Thus, crystallinity either imparted in the copolymer prior to implantation or occurring in vivo over the course of the biodegradation may result in an attendant foreign body reaction which is markedly extended in time due to the retardation of biodegradation by the crystallites.

Winet, Hollinger and Stevanovic have evaluated neoangiogenesis, neoosteogenesis and erosion of 51:49 poly(L-lactide-co-glycolide) threads in an in vivo bone chamber [49]. This study is of interest as the extruded threads measured 100 μm in diameter and thus exhibited a comparable size in two dimensions to biodegradable microspheres. Approximately 50 percent of the poly(DL-lactide-co-glycolide) had degraded by four weeks and complete degradation was achieved in 12 weeks. These authors suggest from the results of these experiments that both delayed angiogenesis and delayed osteogenesis occurred. They suggested that these were secondary consequences of the macrophage response to the eroding poly-L-lactide crystal nanoparticles produced in the degradation products and the influence of reduced nutrient exchange. Neoangiogenesis has not been quantitatively evaluated within the implant sites of biodegradable lactic acid and glycolic acid polymers and copolymers. Since the implants utilized in this study are dimensionally comparable to biodegradable microspheres, these results may suggest that a similar phenomenon is occurring within the implant sites of biodegradable microspheres. A retardation of and reduction in neoangiogenesis within implant sites of biodegradable microspheres may not only have an impact on the bioavailability of any drug that might be incorporated within the biodegradable microspheres but also on tissue responses when biodegradable microspheres are used as matrices in tissue engineering applications.

4.2. Eye

Ogura and Kimura have reported on biodegradable microspheres for targeted drug delivery to the retinal pigment epithelium of the eye [50]. Both 50:50 and 75:25 poly(DL-lactide-co-glycolide) were used for subretinal delivery in rabbits. Retinal pigment epithelium was demonstrated to phagocytose the biodegradable microspheres. Histological evaluation indicated the presence of microspheres at four weeks implantation. In addition, these authors noted that the phagocytosis was enhanced with increasing hydrophobicity of the surface of the biodegradable microsphere. No adverse cellular or tissue reactions were noted in these studies.

4.3. Leuprorelin acetate

Okada and colleagues have carried out extensive investigations of the in vivo degradation and in vivo release profiles of leuprorelin acetate, a potent LHRH analogue, from microcapsules and films prepared from polylactic acids and copoly(lactide/glycolide) [5,51]. Utilizing plates 1 mm in thickness and polylactic acid average molecular weights ranging from 6000 to 50 000 and copoly(lactide/glycolide) average molecular weights from 6000 to 13 500, weight losses were determined over a 100-day period as a measure of biodegradation. In this study, copolymer ratios ranging from 90/10 to 55/45 were implanted in rats subcutaneously. In vivo weight loss profiles were biphasic with a lag time followed by a period when the weight fell exponentially. The lag times and the half-lives of the

degradation increased with an increase in the molecular weight or a decrease in glycolide content. Release of leuprorelin acetate from polylactic acid microcapsules tended to be comparable to the biodegradation rate of the polymer used and the in vivo release of the drug from copoly(lactide/glycolide) microcapsules tended to be comparable to the biodegradation rate of the polymer.

Biocompatibility studies of 75:25 poly(DL-lactic-co-glycolic acid) containing about 8% leuprorelin displayed a minimal inflammatory reaction and a focal foreign body reaction consisting of macrophages and foreign body giant cells. Good biocompatibility with no inflammation and mild angiogenesis within the fibrous capsule was present with the gradual disappearance of the microspheres following bioerosion over a one-month period.

4.4. Central nervous system

The biodegradation of 50:50 poly(DL-lactide-co-glycolide) microspheres, 22 μm average diameter, in brain tissue and the brain tissue reaction have been investigated over a two-month period [52]. The microspheres were prepared by the solvent evaporation method using methylene chloride and were sterilized by exposure to gamma-irradiation. Stereotaxic implantation into the striatum was carried out on Wistar rats and the biodegradation and tissue response were evaluated at one, ten, 21, 30, 60 and 120 days by standard staining techniques, immunohistochemistry and transmission electron microscopy. Within one day, the microspheres were completely surrounded by activated microglial cells and/or macrophages. At ten days, macrophages, occasional foreign body giant cells and astrocytes were present surrounding the microspheres; a similar reaction was seen at 21 days. At one month, the cellular reaction around the microspheres had strongly decreased. At two months, the cellular reaction was not distinguishable with standard staining and only some remnants of the microspheres could be identified. The tissue reaction at four months was comparable to that at two months. It is noteworthy that at two months, isolated macrophages along the needle tract were identified, as at earlier times, but the cellular reaction to the microspheres had almost completely resolved. Immunohistochemical studies were carried out to evaluate the presence of T lymphocytes to address the issue of potential immunogenicity due to the adjuvant properties of the particulate system. No T lymphocytes were observed at any time point at the implantation site.

Tice and colleagues have investigated the effects of dopamine and norepinephrine biodegradable microspheres to reverse the symptoms of CNS neural degenerative disease [53–55]. Injections of 65:35 poly(DL-lactide-co-glycolide) and 50:50 poly(DL-lactide-co-glycolide) microspheres containing dopamine or norepinephrine were injected into two sites of the dopamine denervated striatum of rats previously unilaterally lesioned with 6-hydroxy dopamine. The slower degrading copolymer, i.e., 65:35 poly(DL-lactide-co-glycolide) containing dopamine or norepinephrine displayed the longest acting effects when compared to the 50:50 poly(DL-lactide-co-glycolide) microspheres. Empty control microspheres showed no changes in symptomatology. Immunocytochemical evaluation of the injection sites revealed growth of dopamine and tyrosine hydroxylase immunoreactive fibres in the striatum of dopamine and norepinephrine microsphere implanted rats. No growth of immunoreactive fibres was noted in rats implanted with empty microspheres.

4.5. Nanoparticles and nanospheres

Nanospheres and nanoparticles of PLA and PLGA are generally described as having characteristic dimensions smaller than 10 microns in diameter and thus are available for direct endocytosis or phagocytosis into leukocytes, monocytes, macrophages and other cells of the reticular endothelium system, i.e., liver, spleen, etc. Sites of administration of nanoparticles and nanospheres are direct injection into solid tissues or organs, into body cavities including the intraperitoneal space or pleural space, and intravenously where the nanoparticles embolize to the capillary beds of distant organs such as liver, spleen and lung. Drugs may be incorporated into nanoparticles by three different methods. Drugs may be incorporated within the nanoparticles during the course of the preparation of nanoparticles. In addition, drugs may be adsorbed on preformed nanoparticles or drugs may be chemically bound to the nanoparticles.

Little is known regarding the inflammatory and tissue responses to the injection of nanoparticles.

However, this is an active area of research by numerous groups because of the broad potential of therapeutic application of these materials [56–60].

Intravenous application of nanoparticles, while offering the opportunity to target drug delivery to organs, may also reduce the efficiency of delivery to the target organ through clearance by the reticuloendothelial system (RES) (also called the MPS, mononuclear phagocyte system). Early studies with intravenously injected nanoparticles identified the RES (MPS) as a major barrier to drug targeting as the cells of this system preferentially phagocytosed the blood circulating nanoparticles and markedly reduced the delivery to selected organs. The results of these studies then led to two paths of research activity in an effort to inhibit RES (MPS) uptake of nanoparticles. Efforts have been directed toward understanding plasma protein (opsonin) adsorption to nanoparticles which may facilitate or inhibit phagocytosis by cells of the RES (MPS) [61–64]. Studies are also ongoing to develop stealth coatings for the nanoparticles such that opsonin protein adsorption to the nanoparticles is inhibited and/or phagocytosis by RES (MPS) is inhibited. Both of these issues are important to the biodegradation and biocompatibility of PLA and PLGA nanoparticles.

Opsonins are plasma proteins which adhere to foreign materials and facilitate the phagocytosis of these materials. The two major opsonins are complement and immunoglobulin. The research groups of Gurny and Couvreur have carried out fundamental studies on the role of plasma and serum opsonins on the internalization of biodegradable nanoparticles. Coatings such as polyethylene glycol (PEG) have been demonstrated to protect biodegradable nanoparticles from extensive uptake by leukocytes and monocytes. Other surfactants have been investigated as coatings to inhibit the opsonization process as well as the phagocytosis process.

In an effort to utilize the properties of polyethylene glycol (PEG) in inhibiting protein adsorption and phagocytosis, several groups have incorporated PEG within the molecular architecture of biodegradable polyesters to produce block copolymer systems. Preliminary investigations in the use of these materials for intravenous and intramuscular application are currently underway [65–67].

These fundamental studies offer the opportunity to investigate the phenotypic expression of inflammatory cells following phagocytosis of nanoparticles under various conditions and modifications. A fundamental knowledge of these processes in circulating inflammatory cells as well as RES (MPS) cells within organs will provide new information on perspective modifications of nanoparticles to inhibit the phagocytic process by the RES (MPS).

4.6. Lymphoid tissue

Biodegradable microspheres composed of poly(DL-lactic-co-glycolic acid), 1 to 10 μm in diameter, can be transported into and through Peyer's patches in the gastrointestinal tract, and provide antigens for the development of mucosal or systemic immunity, respectively. Ermak and colleagues have demonstrated that M cells bind and transport microspheres to immunocompetent cells in underlying mucosal lymphoid tissues [68]. Other investigators have found that particles less than 5 μm can be transported through Peyer's patches to peripheral lymphoid organs, whereas particles greater than 5 μm remain within Peyer's patches [69–71]. Administration of biodegradable microspheres which target immune cells within the Peyer's patches may be optimal for initiating mucosal IgA responses whereas biodegradable microspheres which pass through patches may be optimal for the generation of systemic IgG responses. These studies reported no untoward tissue or cellular responses to the biodegradable microspheres within the mucosal epithelium or within the Peyer's patches.

Utilization of biodegradable poly(DL-lactide-co-glycolide) microspheres as an adjuvant for vaccines has been demonstrated by Eldridge, Tice and colleagues [72,73]. As measured by circulating immunoglobulin G (IgG) antitoxin titers, the delivery of Staphylococcal enterotoxin B (SEB) toxoid via poly(DL-lactide-co-glycolide) microspheres, 1 to 10 μm in diameter, induced an immune response which was approximately 500 times that seen with nonencapsulated toxoid when injected subcutaneously in mice. When larger microspheres, 10 to 110 μm, were used, the titer was approximately one-tenth that seen with the smaller microspheres at comparable time periods. Utilizing microspheres with different lactide-to-glycolide ratios, peak IgG titers were approximately the same but the time period to peak IgG titer increased with decreasing glycolide composition. By

utilizing mixtures of microspheres with differing lactide/glycolide ratios as well as different microsphere sizes, these authors demonstrated that lactide/glycolide copolymers could be used for discrete pulsatile vaccine release [72,73].

Similar effects on the modulation of the immune response in mice by varying the composition of the biodegradable copolymer or the particle size have been demonstrated with tetanus toxoid and a synthetic malaria antigen. In this study, Gander and colleagues examined the effect of polymer degradation time, microsphere size, antigen release kinetics and immunological responses in mice [74].

5. Conclusions

Studies to date indicate that PLA and PLGA microspheres containing bioactive agents are biocompatible and when used in therapeutic applications in vivo do not exhibit untoward reactions either locally or systemically. The biodegradation of PLA and PLGA microspheres occurs through a homogeneous hydrolytic chain cleavage mechanism where the rates of polymer degradation are similar for both the surface and the bulk of the microspheres. However, caution is warranted in the application of this principle in the design and development of microsphere controlled release systems as the bioactive agent incorporated into the microspheres may modulate the hydrolytic degradation rate as well as the tissue response. Other factors such as molecular weight and molecular weight distribution as well as sterilization may also alter the degradation rate of the biodegradable polyesters in microspheres.

The rate of degradation as well as the localized tissue response to microspheres is size-dependent. Microspheres greater than 5 to 10 microns in diameter may not be phagocytosed within macrophages and foreign body giant cells and thus a foreign body reaction is present at the surfaces of these microspheres. Microspheres smaller than 5 microns may undergo phagocytosis by macrophages, foreign body giant cells and other types of cells. Incorporation of these smaller microspheres and nanoparticles or nanospheres may lead to a more rapid degradation of the biodegradable polyester. Regardless of composition, the foreign body reaction with the presence of macrophages and foreign body giant cells is a common tissue response to microspheres greater than 10 microns in diameter. This reaction is a part of the tissue response continuum and follows the early response and acute and chronic inflammation secondary to implantation or injection injury.

Microspheres composed of PLA and PLGA have proven useful in providing local responses such as the facilitation of rapid bone growth through the use of bone morphogenetic protein or through systemic responses by the release of leuprorelin.

New areas of application, such as the application of PLA and PLGA as controlled release systems in the brain and the development of nanospheres for intravenous application are currently under investigation. Fundamental studies on the opsonization process, i.e., blood protein adsorption to nanosphere surfaces, will provide new insights into the modulation and control of cellular responses and the phagocytic process with nanospheres. Insight into these mechanisms will also facilitate and broaden the application of PLA and PLGA microspheres and nanospheres for vaccine development.

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