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BIODEGRADABLE POLY(ESTERS) FOR THE CONTROLLED DELIVERY OF VACCINES

by

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BACKGROUND

Biodegradable polymers have a variety of potential biomedical applications in addition to their current use as suture material [1]. Biodegradable polymers used in controlled release applications are primarily insoluble and undergo chemical hydrolysis to soluble monomeric or oligomeric units [2]. The major advantages of biodegradable polymers are that they do not require removal after delivery of bioactive agent is complete. Additionally adverse tissue reactions from implanted polymer may be ameliorated as the polymer degrades [3]. However, because these polymers degrade with time, their removal is often difficult.

Recently, there has been interest in developing new methods to deliver both vaccines and adjuvants. Often delivery of an immunogen or accompanying adjuvant must be delayed or sustained over a prolonged period of time to heighten the immune response eliminating the need for boosters. Appropriate presentation of the vaccine to the immune system may also be required, particularly for highly purified antigens prepared by recombinant technology.

Poly(esters) were chosen for a study as a vaccine delivery vehicle because they are currently widely used in suture materials and have demonstrated low toxicity. In recent studies we have prepared polyester microspheres by spray-drying [4]. We demonstrated that these poly(ester) microspheres degrade in four major stages: 1. Polymer hydration, 2. Strength loss caused by breakage of backbone bonds in polymer, 3. Loss of mass integrity, and 4. Solubilization [5]. Since poly(ester) matrix biodegradation generally involves bulk erosion, release takes place prior to matrix biodegradation under diffusion-control. This has created problems in the delivery of drugs where zero-order release kinetics is often desirable [2,6]. Precise control of release kinetics may not be as important as delayed or sustained release in the application of these polymers to vaccine delivery. The need to remove a vaccine-containing device before an entire dose is delivered is not anticipated, eliminating the difficulty of recovering biodegradable microspheres. Earlier studies examined different poly(ester) matrices to obtain an ideal polymer life-time. When microspheres of these polymer matrices were prepared containing protein, they released their entire dose rapidly within one week [4,5]. This release profile was not appropriate for vaccine delivery applications.

This research focuses on microspheres prepared form three biodegradable poly(esters) as potential carriers of recombinant proteins for vaccine use. Experiments show matrix life-times compatible with vaccine applications. Release studies demonstrate a delay of up to three weeks is possible making these formulations potentially useful for vaccine applications.

MATERIALS AND METHODS

Poly(lactide:glycolide, 50:50 & 85:15) were from Birmingham Polymers. Poly (L-lactide), molecular weight 50,000 was from Polysciences, Inc. Polymers were used either directly as powders, as particles or were spray-dried from a 1-10 (w/v)% solution in methylene chloride at 25°C using a Yamato Pulvis Min-Spray GA-32 spray drier. The particles formed by spray drying were measured by SEM.

Polymer molecular weight was determined by dissolving the polymer at 0.5 (w/v)% in CH_2Cl_2 or THF and injecting 50 µl onto a Ultrastyragel® (mixed bed) column at 1 ml/min, 25°C with RI detection. The column was calibrated using polystyrene molecular weight standards (Polyscience). Viscosity was measured in CH_2Cl_2 at 25°C using a Cannon-Ubbelohde viscometer.

Biodegradation studies were conducted by placing polymer (200 mg) into 2.5 ml of PBS, and shaking at 37°C. Biodegradation studies were performed at 85°C without agitation. Soluble products were obtained by periodically removing all the PBS from the vessel containing polymer and replacing it with the same volume of fresh PBS [8]. The polymer residue was recovered, washed with water and dried under vacuum and weighed.

Protein release was measured from poly(lactide:glycolide, 50:50) microspheres (5 micron) containing 5% BSA, prepared by suspending 50 mg of spray-dried BSA in 50 ml CH_2Cl_2 containing 1 g of dissolved poly(ester) and then spray drying. Microspheres (200 mg) were placed in 2.5 ml of PBS at 37°C. No surfactant was added to the release medium which led to beads which aggregated during the release/hydrolysis study. Each day the PBS was completely removed and the protein content was measured using Bradford's method [9]. Fresh PBS (2.5 ml) was then added to replenish that which had been removed.

RESULTS AND DISCUSSION

Microspheres of all three poly(esters) were prepared by spray drying [4,5,8] with and without a 5% loading of BSA [4,5]. Polymer microspheres without BSA were analyzed for mass loss as a function of time (Fig 1,2 & 3a). Both 50:50 and 85:15 copolymers (Fig 1 & 2a) showed complete mass loss in PBS at 37°C over a period of 40 and 90 days, respectively. The more crystalline poly(L-lactide) showed no mass loss over a period of 150 days. (Fig 3a). These results suggest that the copolymers might be most useful for applications where the polymer matrix must be eliminated within a short time following immunization.

In addition to examining the mass loss that occurred as a function of time, changes in polymer viscosity and molecular weight (measured by GPC) were also examined (Fig 1,2 & 3, b & c). At various time intervals, the polymer microspheres were removed from the PBS, washed, dried, dissolved in CH_2Cl_2 and assayed. In all three polymers both decrease in viscosity and decrease in molecular weight was observed as a function of time. It is particularly interesting to note that even though no mass loss was observed in poly(L-lactide) over the time frame of these experiments, significant changes in both polymer viscosity and molecular weight were detected (Fig 3b & c). These results suggest that polymer chain scission begins to take place as soon as the polymer is exposed to PBS but that even over a period of 70 days the polymer molecular weight is not reduced sufficiently to permit mass loss through solubilization.

Protein release studies were conducted using microspheres of the three polymers each containing a 5% loading of BSA (Fig 4 a,b,c). Protein release was measured using Bradford assay

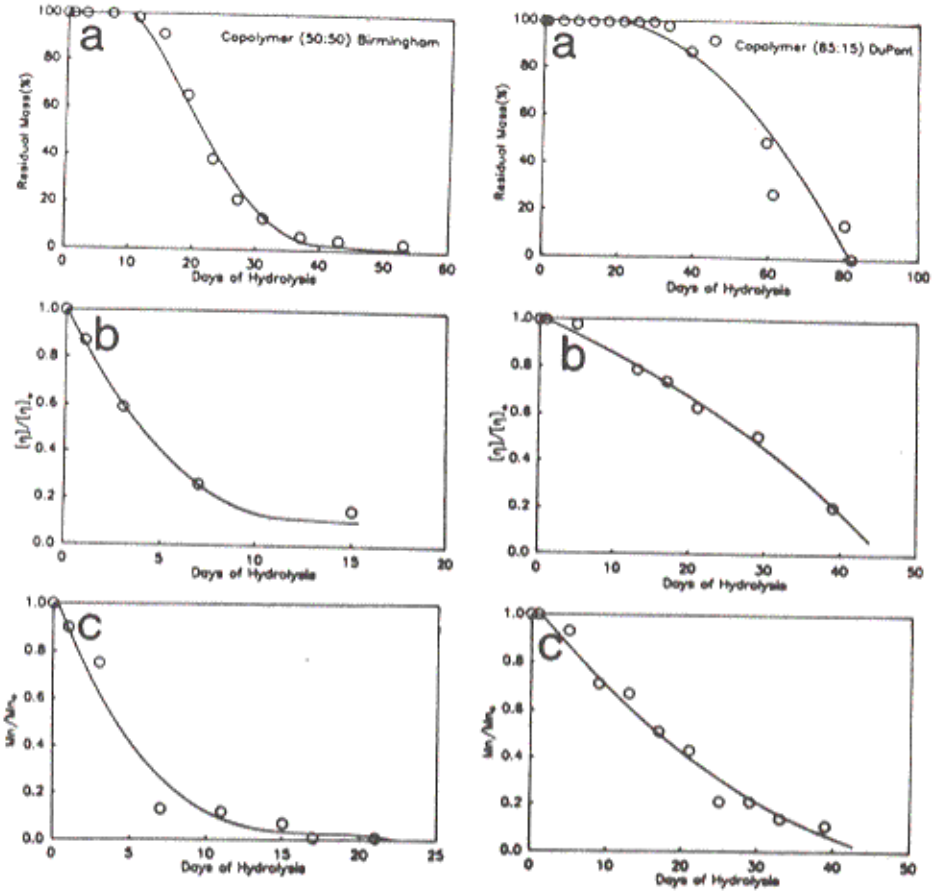

Figure 1 and 2. Hydrolysis of copolymer 50:50 (left panels) and copolymer 85:15 (right panels). The residual mass (a), change in viscosity (b) and change in Mn (c) are plotted as a function of time in days.

[9] and the identity of the BSA found in the PBS release media was confirmed using SDS polyacrylamide gel electrophoresis. The results of these studies demonstrate that protein release is delayed over a period of from 12 to 26 days depending on the polymer matrix that is used.

The time between administration of a vaccination and booster ranges from 2-4 weeks suggesting that the approaches described in this paper will be useful for the effective controlled delivery of vaccines. Future studies are focussing on further delaying the release of proteins by the application of a polymer barrier coat to the microsphere formulations.

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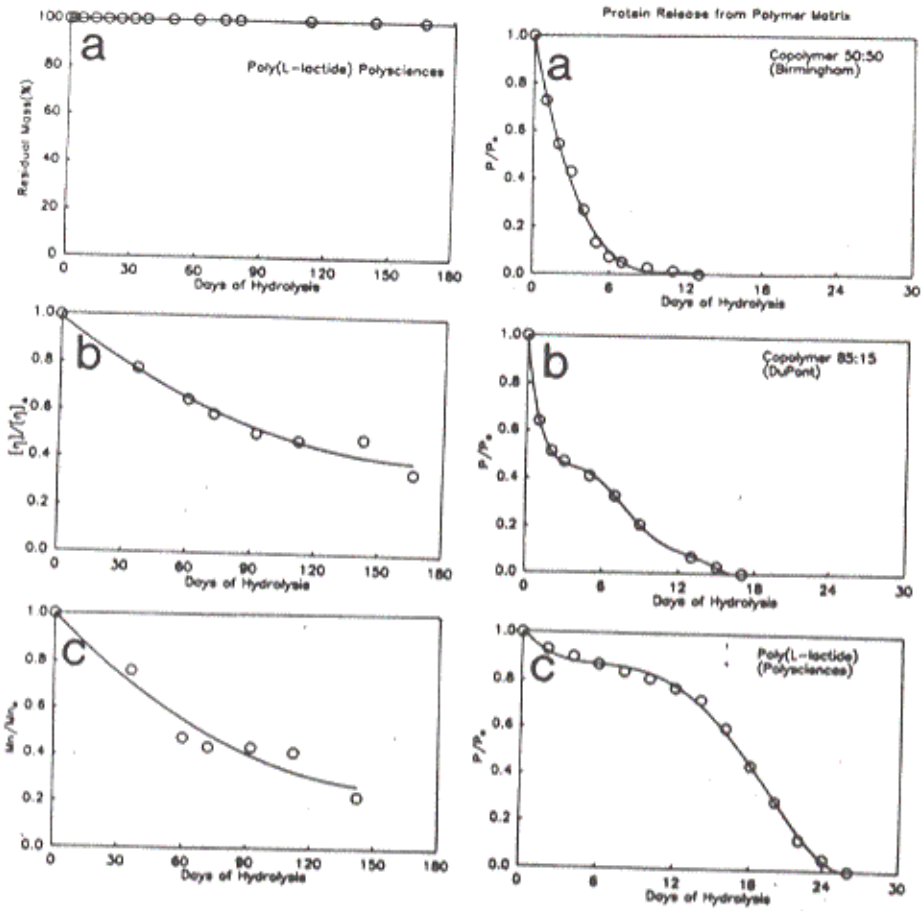

Figure 3 and 4. Hydrolysis of poly (L-lactide) (left panel) is followed by measuring the residual mass (a), change in viscosity (b) and change in Mn (c) as a function of time in days. Protein release is shown in the right panel. The fraction of protein remaining in the copolymer 50:50 (a), copolymer 85:15 (b) and poly(L-lactide) (c) is plotted as a function of time in days.

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