BIODEGRADABLE POLYMERS FOR THE CONTROLLED DELIVERY OF VACCINES

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New methods for the efficient and effective administration of vaccines are required particularly with the advent of new synthetic subunit vaccines. This chapter describes the approaches of several research groups to administer vaccines using biodegradable polymer carriers. Delivery systems composed of synthetic poly[esters] and poly[iminocarbonates] and natural (crosslinked serum albumin) biodegradable polymers are described. The antibody levels in response to these systems are presented, and possible mechanisms responsible for the observed effects are discussed. The important questions that need to be answered before this technology can be successfully applied are also discussed.

INTRODUCTION

Biodegradable polymers have been used for a variety of medical applications including the controlled release of drugs and biologicals.[1] One potential application of biodegradable polymers is the controlled delivery of vaccines. Worldwide, vaccinations represent hundreds of millions of human treatments annually.[2] One problem with traditional vaccinations is the need for repeated booster regimens. Controlled release may obviate the need for such regimens. This could be a substantial advantage in situations where compliance makes it difficult to administer repeated injections. Additionally, many new synthetic subunit vaccines are insufficiently immunogenic alone to be successful and thus require the use of adjuvants and or new delivery techniques.[3] Current adjuvants such as aluminum hydroxide and Freund's complete and incomplete adjuvants are believed to exert as least part of their effect by prolonging the release of antigen from the injection site into the surrounding tissues. Our laboratory has shown that a wide range of potential *in vitro* release profiles may be achieved by careful selection of the polymer and formulation method.[4,5] Biodegradable and biocompatible microspheres or pellets seem well suited to provide slow or delayed release of antigen without the undesirable side effects of other adjuvants. Therefore, the modifica-

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tion of release kinetics or incorporation of both vaccine and adjuvant into biodegradable polymers may substantially improve the immunogenicity of many new vaccines.

INITIAL STUDIES

As early as 1976, Chang described the use of poly(lactic acid) for the microencapsulation of vaccines and antigens but did not study the release properties or immunogenicity of these formulations.[6] Preis and Langer later showed that the sustained release of bovine serum albumin, ribonuclease-A and bovine γ-globulin from ethylene-vinyl acetate pellets (0.3 mm^3) resulted in sustained antibody levels in mice for 25 weeks.[7] The antibody (IgG) levels in mice (n = 8) implanted with a single BSA/polymer pellet (50μg BSA) were comparable to those in mice (n = 4)

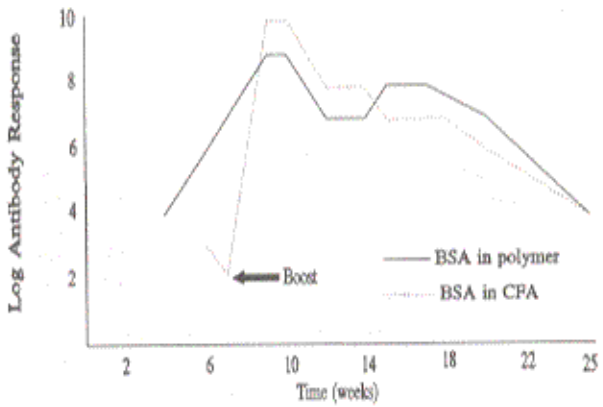

Figure 1. Antibody response to BSA in CFA given in two doses compared to sustained release from a polymeric matrix. (Prepared from data in reference 7.)

given a primary injection of BSA (50 μg) in complete Freund's adjuvant (CFA) followed by an identical booster regimen seven days later (100 μg total BSA) (Figure 1). These results gave the impetus for further study since they showed that sustained release of antigens from polymeric matrices could indeed elicit a substantial and prolonged immune response with a single treatment. In a second set of experiments these authors studied the ability of 3 different antigens of varying molecular weight to induce an immune response when given as antigen/polymer pellets. Bovine γ-globulin, bovine serum albumin and ribonuclease-A having molecular weights of 158,000, 68,000 and 14,000, respectively, all elicited a prolonged immune response. The ethylene-vinyl acetate pellets used in this study, however, do not degrade *in vivo* and thus would require surgical removal of the residual polymer making it less convenient than traditional inoculations. These experiments did however show that the sustained release of vaccines and adjuvants from polymeric matrices was a viable route for single-step immunization.

SYNTHETIC BIODEGRADABLE POLYMERS

Kohn, et al. studied an antigen delivery system based on poly(CTTH-iminocarbonate) (IUPAC nomenclature: poly-[oxyimidocarbonyloxy-p-phenylene-[2-(hexyloxycarbonyl)-ethylene]imino-[2-[1-(benzyloxy)-formamido]-1-oxotrimethylene]-p-phenylene]).[8] This polymer was designed with the aim that its primary degradation product, N-benzyloxycarbonyl-L-tyrosyl-L-tyrosine hexyl ester (CTTH) would show adjuvant properties as was shown for other L-tyrosine derivatives by Miller and Tees.[9] Indeed, the authors found CTTH to be as potent an adjuvant as Freund's complete and muramyl dipeptide (MDP).[8] *In vitro* release profiles appeared to follow a square-root of time dependence with most of the antigen released within 25 days (Figure 2). The square-root of time dependence and the observed effect of loading on release kinetics suggested that the release of antigen was controlled by a matrix diffusion mechanism and not determined by the degradation of the polymer. *In vivo* studies were carried out in mice using eight treatment groups of ten mice each. The treatment groups were as follows:

GROUP	TREATMENT
A	25 μg BSA in 1 mL physiological saline solution (PSS), boosted at 4 weeks
B	25 μg BSA in 1 mL CFA (1:1 emulsion), boosted at 4 weeks
C	25 μg BSA + 100 mg MDP in 1 mL PSS, boosted at 4 weeks
D	25 μg BSA adsorbed onto 40 mg of solid tyrosine in 1 mL PSS, boosted at 4 weeks
E	25 μg BSA adsorbed onto 40 mg of solid dityrosine in 1mL PSS, boosted at 4 weeks
F	25 μg BSA adsorbed onto 40 mg of solid CTTH in 1 mL PSS, boosted at 4 weeks
G	50 μg BSA incorporated in a poly(BPA-iminocarbonate) device
H	50 μg BSA incorporated in a poly(CTTH-iminobonate) device

The devices implanted in groups G and H were 0.5 mg films cast from CH_2Cl_2 solutions containing suspended BSA. The anti-BSA antibody titers after 56 weeks were over twice as high in animals given a single injection of the poly(CTTH-iminocarbonate) formulation (Group H) compared to animals given BSA at 0 and 4 weeks. (Group A) (Figure 3)

Within the last few years, many researchers have been evaluating the polyesters- and copoly(esters) of lactic acid and glycolic acid as matrices for the delivery of peptides and proteins including vaccines and antigens. These polymers have already been used in humans for many years as surgical sutures and have well established biocompatibility and biodegradability. Thus, their regulatory status is well established. Also, a wide range of potential degradation times from a few weeks to over 1 year are attainable by selecting the proper copolymer and molecular weight. Miller, et al. have shown the influence of copolymer composition on the degradation time for copolymers of L-lactic and glycolic acids (Figure 4).[10] These polyesters have comparatively well characterized degradation profiles and are known to degrade by bulk hydrolysis. This means that if release is degradation-controlled, a biphasic release profile is expected for molecules, such as proteins, unable to diffuse through the intact polymer. A typical *in vitro* release profile for a model compound in shown in Figure 5. The model compound, amaranth, is a hydrophilic dye with three sulfonate groups which has been shown not to diffuse through the poly(D,L-lactide-co-glycolide, 50:50) polymer used.

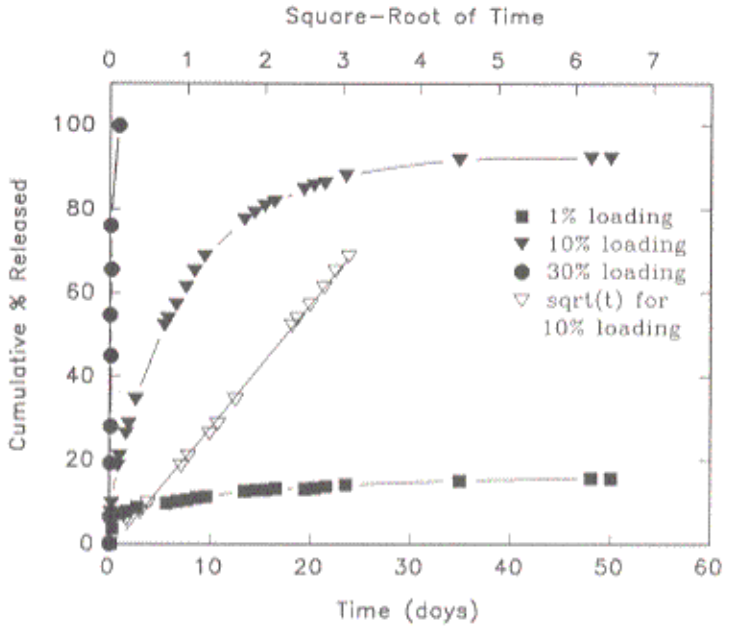

Figure 2. *In vitro* release profiles of Eosin Y from poly-(CTTH-iminocarbonate) devices containing different loadings. (Prepared from data in reference 8.)

This type of release profile may prove useful since it closely approximates the traditional release kinetics seen with an initial dose followed by a booster regimen.

O'Hagan, et al. studied the immunogenic response of mice to ovalbumin (OVA) entrapped in poly(D,L-lactide-co-glycolide, 50:50) microparticles by two routes of immunization, subcutaneous (s.c.) and intraperitoneal injection (i.p.).[11] The microparticles were formed from the copolymer (MW = 9000) by an oil-in-water emulsion method. Characterization showed the microparticles to have an average loading of 1% w/w OVA and a volume-mean diameter of 5.34 μm. Antibody (IgG) levels in response to injections of soluble ovalbumin, ovalbumin emulsified in CFA and ovalbumin entrapped in poly(ester) microcapsules were measured. Both primary and secondary responses were measured. Ovalbumin is a rather poor immunogen thus the results of this study were useful in assessing the adjuvant effect of the microparticulate delivery system. The results for the primary immune response showed that after single i.p. injections (100 μg OVA), the mice given encapsulated OVA had a significantly greater IgG titer than both the primary and secondary responses to soluble OVA throughout the 12 week study. The responses to the encapsulated OVA were also significantly greater than the OVA/CFA preparation for 10 weeks following injection. At 12 weeks, however, the two treatment groups were not significantly different. The adjuvant effect of the microparticulate formulation was proposed to be due to the uptake of small particles by macrophages which then present the antigen. Thus particle size would be very important for this mechanism. The previous results of Langer, et al., which used 0.3 mm^3 pellets however, do not seem to show this same importance of particle size since their delivery system was found to be nearly as effective as a CFA emulsion even though the pellets were much too large to be phagocytized by macrophages.[7] The secondary response was

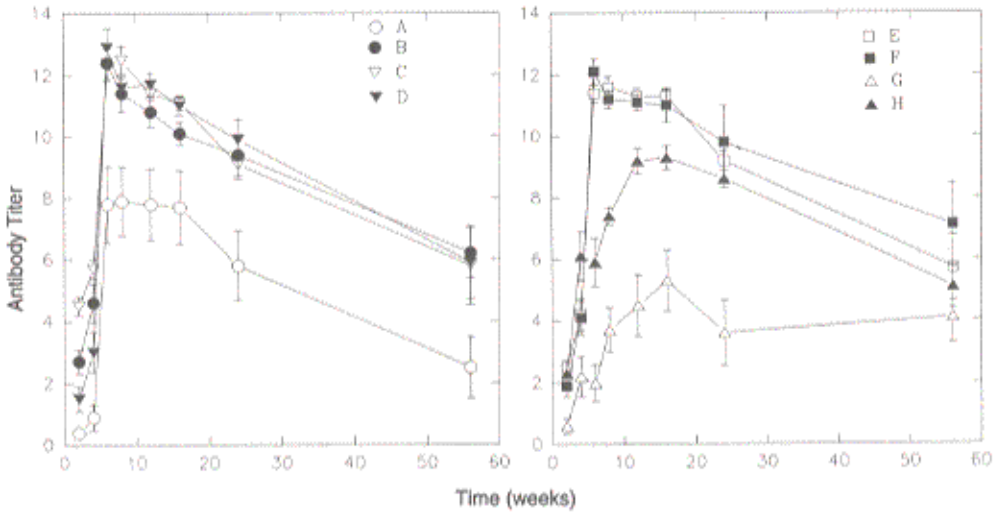

Figure 3. Mean hemagglutination BSA antibody titers as a function of time for the eight treatment groups used by Kohn et al. Data represent the mean of ten animals ± SEM. (Prepared from data in reference 8.)

evaluated using three groups of ten mice each. The treatment groups were given primary s.c. injections of (1) soluble OVA, (2) encapsulated OVA, or (3) OVA emulsified in CFA followed by booster injections at six weeks. The booster injections were identical to the primary injections except for group (3) where the booster was OVA dispersed in Freund's incomplete adjuvant (FIA). The difference between the encapsulated OVA and OVA/CFA formulations were not significant for the secondary response but both showed a greater response than was achieved with soluble OVA. In a subsequent publication the authors reported that OVA entrapped in poly(lactide-co-glycolide) microspheres gave a significantly lower response than OVA/CFA when studied in rats.[12] This was true for both the primary and secondary responses. The microsphere formulations used in these two studies were from the same batch. The only difference between the two experiments was the animal model. The first study used the mouse and the second used the rat. The authors addressed this disparity but were unable to offer any explanation for the observed results.

Eldridge, et al. found that poly(D,L-lactide-co-glycolide) microspheres represented a potent adjuvant system for Staphylococcal enterotoxin B (SEB) capable of inducing both circulating and mucosal immunity when given orally.[13] Eldridge, et al. also found that orally administered poly(D,L-lactide-co-glycolide) 1-10 μm microspheres containing SEB were specifically taken up into the Peyer's patch lymphoid tissue of the gut.[14] Furthermore, SEB- containing microspheres induced circulating anti-SEB antibodies and a secretory IgA anti-SEB response in saliva and gut fluid, while soluble SEB did not. These results suggest that the polymer was able to protect the antigen from the gastrointestinal environment. In another study, Eldridge, et al. evaluated the immune response of mice given SEB-containing poly(D,L-lactide-co-glycolide) microspheres subcutaneously.[15] The results showed that the kinetics, magnitude and duration of the immune response to encapsulated SEB were similar to those obtained after an equivalent dose emulsified in CFA

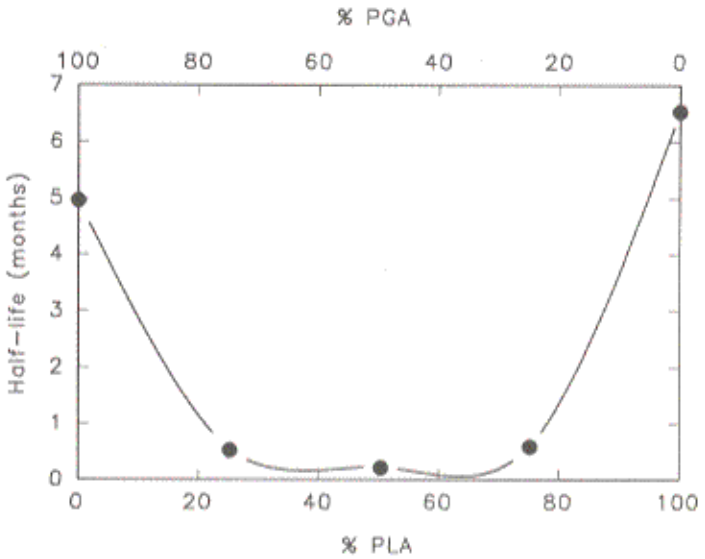

Figure 4. Degradation half-life for copolymers of L-lactic acid and glycolic acid as a function of copolymer composition. (Prepared from data presented in reference 10.)

(Figure 6). The microspheres had the additional benefit that they did not induce the inflammation and granulomata observed when CFA was present. Antigen-containing microspheres 1 to 10 μm in diameter exhibited stronger adjuvant activity than those >10 μm suggesting that uptake of the microspheres by macrophages is an important factor in the adjuvant activity thus supporting the conclusions of O'Hagan, et al.[11]

Beck, et al. have described a system for delivering antigens and antibodies to the female reproductive tract.[16,17] In these studies Pneumococcus bacteria, herpes simplex viral antigens and bovine chorionic gonadotrophin hormone (HCG) were microencapsulated with lactide/glycolide polymers. The *in vivo* effects of vaginally administered microcapsules were followed in rabbits. The results of vaginal washes showed that immunization was successful at two weeks post treatment. This showed the primary response to be intact however no data was given for the secondary response. Workers at Stolle International have described a delivery system designed to release antigen to dairy cattle over 6 to 12 months.[18] Animals were injected intramuscularly with antigen-containing microparticles suspended in an aqueous vehicle. The antibody titers in milk of animals given a single dose of microparticles were as high as those found in animals after conventional immunizations which had the inconvenience of requiring several booster injections.

Our laboratory has recently formulated a biodegradable microparticulate vaccine preparation by a coacervation process using poly(D,L-lactide-co-glycolide, 50:50) which is currently undergoing *in vivo* evaluation. (Figure 7)

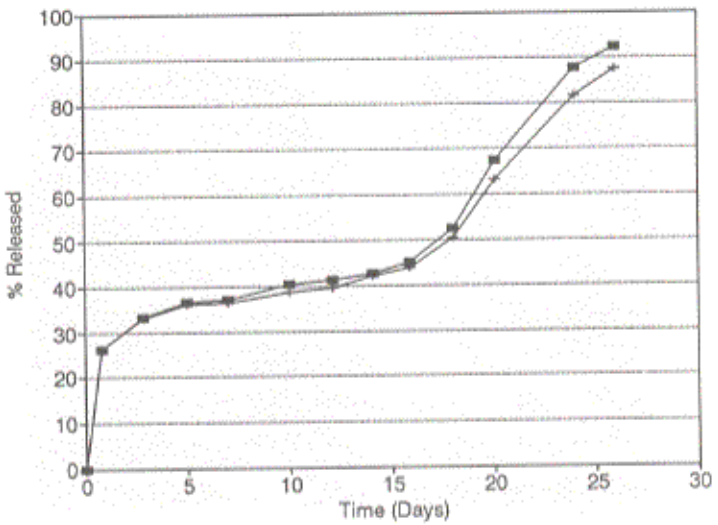

Figure 5. Release of amaranth in phosphate buffered saline at 37°C from poly(D,Llactide-co-glycolide, 50:50) microparticles prepared by a coacervation method.

NATURAL BIODEGRADABLE POLYMERS

Lee, et al. first reported the preparation of solid albumin microspheres produced by mild chemical crosslinking of serum albumin with glutaraldehyde.[19] They found that the *in vitro* release profiles of steroids from these systems could be varied by controlling the cross-link

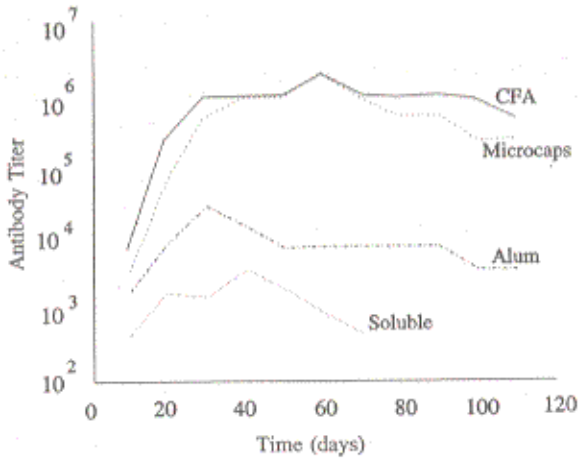

Figure 6. Antibody response to SEB toxin formulations. (Prepared from data presented in reference 15.)

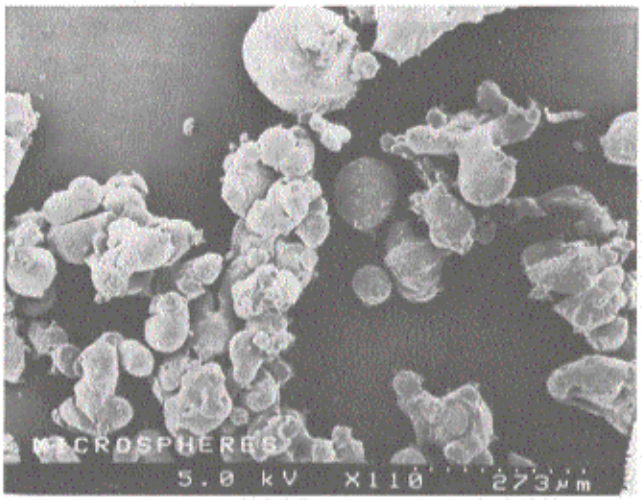

Figure 7. Scanning electron micrograph of a poly(D,L-lactide-co-glycolide, 50:50) vaccine formulation prepared by coacervation.

density. The *in vivo* degradation of these microspheres would proceed via endogenous proteases. The method of Lee et al. was subsequently used by Dewar, et al. to entrap virus particles.[20] The model virus employed, Nodamura virus, a small, nonpathogenic, RNA virus was entrapped in rabbit serum albumin (RSA) beads and later studied *in vivo* in rabbits. It is important to mention that proteins entrapped into albumin beads in this way would be covalently bound to the albumin and potentially to other protein molecules. The purpose of the study was to determine whether the formulation procedure or the release characteristics had any deleterious effects on the antigenicity of the virus. The results showed that the viral antibody titers in rabbits given a single injection of the RSA bead formulation paralleled those given a single injection of the virus in CFA for 60 days, the duration of the study. This suggested that the RSA bead formulation possessed an adjuvant action approximating CFA, presumably due to slow release of the virus from the protein matrix since the beads were too large (100-200 µm) to be phagocytized by macrophages. Continuing this work, Martin, et al. studied the effectiveness of serum albumin beads as a delivery system for subunit vaccines.[21] The 40 kdalton Nodamura capsid protein was used as a model. The antibody responses to the capsid protein and the whole virus given in RSA beads were slow compared to soluble vaccines. The entrapped vaccines showed a continuous increase in the levels of circulating antibodies which, at 60 days post-injection, was approximately equal to the peak responses obtained with soluble vaccines. The authors were unable to show any antigenic properties of the empty beads in homotypic animals.

Langhein, et al. studied the effectiveness of different antigenic materials entrapped in the same way in albumin beads.[22] Model vaccines containing *Clostridium botulinum* type D toxin and *Klebsiella pneumoniae* capsular polysaccharide antigen were prepared. The *in vivo* evaluation of this system employed four treatment groups: (1) *Cl. botulinum* toxoid; (2) *Cl. botulinum* toxoid emulsified in Freud's incomplete adjuvant; (3) *Cl. botulinum* toxin covalently bound into RSA beads; and (4) Cl. botulinum toxin bound into RSA beads and stored for 4 months at room temperature.

The stored formulation retained nearly all of its immunogenic activity. This finding could be of practical importance since the formulation of a dry, stable vaccine could reduce storage and transportation costs.

The synthesis of cross-linked starch, a second type of natural biodegradable polymer, has been described by Artursson, et al.[23] To date, this polymer has been applied to the delivery of enzymes to intracellular compartments of the reticuloendothelial system after intravenous injection.[24] The authors found the poly(acrylstarch) microparticles to be potent adjuvants in testing the tendency of the particles to induce an autoantibody response to autologous proteins.[25,26] These polymers, however, have not yet been applied to vaccine delivery systems.

There seems to be a potential problem with these naturally derived biodegradable polymers which is related to their enzymatic degradation mechanism. Since the presence or concentration of some enzymes can vary substantially among individuals, in addition to interspecies variability, the rate of matrix degradation and thus the rate of vaccine release could vary considerably among different patient or animal populations. Synthetic biodegradable polymers such as the poly(esters), poly(iminocarbonates), poly(anhydrides)[27] and poly(orthoesters)[28] were designed to degrade through chemical hydrolysis in water and should show less biologic variability.

FUTURE APPROACHES

In the future, the mechanism by which vaccines encapsulated in biodegradable polymers induce immunization requires study. The size of the delivery system may be of paramount importance as suggested by O'Hagan, et al.,[11] or it may only play a small role as would be suggested by the data of Langer and Preis.[7] Alternatively, the release kinetics may determine the magnitude of the effect. Ideal release kinetics may not be zero-order or matrix-controlled but instead may be pulsatile mimicking standard immunization protocols. The answer to these questions will largely determine the direction taken to formulate these systems. The optimization of vaccine delivery using biodegradable polymers will require that these fundamental questions be answered. This may suggest the use of alternate biodegradable polymers having adjuvant activity or the novel use of existing materials such as the lactide/glycolide polymers. Continued research should address these important questions.

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