The use of poly(D,L-lactic-*co*-glycolic) acid microspheres in the treatment of allergic disease

William R. Reisacher and Dara Liotta

Department of Otorhinolaryngology, Weill Cornell Medical College, New York – Presbyterian Hospital, New York, New York, USA

Correspondence to Assistant Professor William R. Reisacher, MD, FACS, FAAOA, Department of Otorhinolaryngology, Weill Cornell Medical College, 1305 York Avenue, 5th Floor, New York, NY 10021, USA
Tel: +1 646 962 2093; fax: +1 646 962 0100; e-mail: wir2011@med.cornell.edu

Current Opinion in Otolaryngology & Head and Neck Surgery 2011, 19:188–192

Purpose of review

This article reviews literature on the use of poly(D,L-lactic-*co*-glycolic) acid (PLGA) microspheres for the treatment of IgE-mediated hypersensitivity. The discussion will focus on the encapsulation and release of proteins from microspheres, the interaction of microspheres with the immune system through different routes of delivery, and the therapeutic changes that are noted following this form of allergy immunotherapy.

Recent findings

Allergenic proteins which are encapsulated into PLGA microspheres are able to be recognized by the immune system. When placed into the body via injection or ingestion, the proteins are slowly released from the microspheres over an extended period of time, producing a shift in the immune system away from an allergy-dominant pattern. This has been shown to cause an improvement in symptoms, a decrease in medication use and protection against developing severe allergic reactions.

Summary

Because of the slow release of protein from the microspheres over time, less frequent treatments are necessary compared with conventional immunotherapy, resulting in a more convenient treatment. Because regular bolus injections are avoided, a steadier level of protein is delivered to the body, and this may result in a safer, as well as more effective, form of immunotherapy.

Keywords

allergy, antigen, hypersensitivity, immunotherapy, microspheres, poly(D,L-lactic-*co*-glycolic) acid

Curr Opin Otolaryngol Head Neck Surg 19:188–192
© 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins
1068-9508

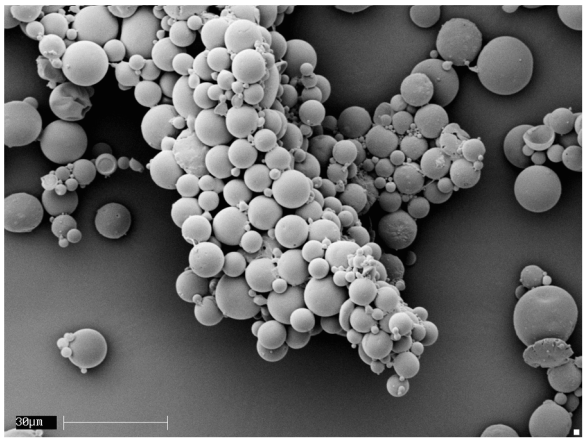
Introduction

Absorbable polyesters have been used for multiple medical purposes, including sutures, bone fixation devices, tissue engineering scaffolds and controlled drug delivery systems [1••]. One such polymer is poly(D,L-lactic-*co*-glycolic) acid (PLGA), which is composed of a backbone of polyglycolide and polylactide. PLGA is highly biocompatible as well as biodegradable. When coming into contact with artificial or biologic fluids, it is broken down by nonenzymatic hydrolytic cleavage into lactic and glycolic acids, which are further metabolized into carbon dioxide and water [2]. Microscopic spheres (microspheres) made from PLGA can be loaded with a variety of proteins and are able to slowly release this protein over time (Fig. 1). Protein-loaded PLGA microspheres were first introduced in the mid-1980s for the purpose of cancer therapy [3]. Since that time, they have been used for the delivery of chemotherapeutic drugs, immunosuppressants, anti-inflammatory agents, antibiotics, opioid antagonists, steroids, hormones, anesthetics, vaccines and gene therapy [4]. Microspheres have also been used to deliver photosensitizing agents for photodynamic treatment of head and neck cancer [5•].

Recently, PLGA has been investigated as a possible vehicle for the delivery of antigen-specific immunotherapy, which is considered the 'gold standard' of treatment for IgE-mediated environmental allergies. Antigen-specific immunotherapy, via the subcutaneous route (SCIT), involves the weekly injection of increasing levels of allergenic proteins over a period of 3–5 years. Developed by Noon in 1911, this therapy shifts the immune system from the T helper (Th)2-dominated, allergy-producing pattern toward the Th1-dominant pattern. SCIT provides significant improvement of total symptom scores and quality of life scores in patients with allergic rhinitis and decreases the need for medication use [6]. However, several barriers to the acceptance of this therapy exist, including the risk of anaphylaxis, the inconvenience of weekly office visits, and the aversion to repeated injections, particularly in children.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

Figure 1 Original photomicrograph of poly(D,L-lactic-*co*-glycolic) acid microspheres



The microspheres studied at low voltage (5 kV) using scanning electron microscopy imaging (LEICA 440) after coating with palladium. Timothy grass (*Phleum pratense*) pollen protein is contained within each microsphere.

It is the ability of the microspheres to slowly deliver proteins over time that raised interest in their use in allergic disease. Less frequent therapy may enhance patient acceptance as well as the efficacy and safety of the treatment. Pharmacokinetic data suggest that the success of immunotherapy is not related to the rapid absorption of large amounts of allergenic protein into the systemic circulation, but rather the retention of lower doses in the submucosal and subcutaneous spaces for an extended period of time in order to stimulate lymphoid tissue [7]. By avoiding a weekly bolus, the protein spends more time in the therapeutic window, thus avoiding unwanted reactions associated with rapidly elevated levels.

Poly(D,L-lactic-*co*-glycolic) acid microspheres and allergenic proteins

In 1994, Sharif *et al.* [8,9] first encapsulated dust mite allergen, Der p 1, into PLGA microspheres using a double-emulsion technique. They determined that the encapsulation and release processes did not significantly alter the ability of the protein to bind to IgG and IgE, making it a feasible strategy for desensitization. Seven years later, Iguarta *et al.* [10] reported on the similar stability of olive (*Olea europaea*) pollen, Ole e 1, using electrophoresis to detect protein fragments. In 2002, Guerin *et al.* [11] reported that the encapsulation of bee venom phospholipase A2 (PLA2) into PLGA microspheres had only a limited effect on the integrity of the protein, which retained its specific murine IgE-binding capacity in full. The results suggested that microspheres could be an effective delivery system for administration of bee venom PLA2.

Key points

- Poly(D,L-lactic-*co*-glycolic) acid (PLGA) is a highly biocompatible and biodegradable polymer which currently has many uses in the medical field.
- Allergenic protein is able to be encapsulated and released from PLGA microspheres without being significantly altered.
- Allergenic protein released from PLGA microspheres is able to shift the immune system response from a Th2-dominant profile toward a Th1-dominant profile.
- PLGA microspheres may be administered through different routes of delivery, including oral, nasal and subcutaneous.
- There is evidence that antigen-specific immunotherapy can provide a therapeutic benefit in both mice and humans and may also represent a safer, more convenient treatment than currently available strategies.

The next step for allergen-encapsulated microspheres was determining their ability to shift the immune system toward a Th1-dominant pattern. In 2003, Batanero *et al.* [12] immunized mice with four intraperitoneal injections of either Ole e 1 adsorbed to the immunostimulant $Al(OH)_3$ or Ole e 1 encapsulated into PLGA microspheres. High levels of antigen-specific IgG2a (characteristic of a Th1 response) were found in the serum of mice treated with Ole e 1 microspheres, whereas high levels of IgG1 and total IgE were found in the Ole e $1 + Al(OH)_3$ group. Interferon (IFN)-γ was detected in the supernatant cultures of splenocytes from the mice treated with Ole e 1 microspheres, whereas interleukin (IL)-4 was detected in the group receiving Ole e $1 + Al(OH)_3$, further supporting a shift toward the Th1-dominant pattern in mice receiving microspheres.

It is possible to control the release rate of encapsulated protein and the tissue delivery target by varying the size of the microspheres or by changing the polymer [13]. The release pattern from microspheres includes a burst phase followed by a steady release of protein [14]. Encapsulated Ole e 1, 2-µm microspheres released 15% of their contents during the first day due to protein adsorbed onto the microsphere shell, whereas 95% of the encapsulated protein was released over 38 days from the channels and pores of the degrading inner matrix [10]. Microspheres as small as 1 µm in diameter are able to produce an immune system response [15]. Subcutaneously injected microspheres less than 10 µm are able to be taken up by macrophages, whereas larger microspheres remain in the region, releasing protein [16,17]. Orally ingested microspheres less than 10 µm in diameter are taken up by macrophages in Peyer's patches, whereas larger microspheres are not absorbed

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

in the gastrointestinal tract. Microspheres less than 5 μm are then transported to mesenteric lymph nodes and the spleen, whereas microspheres between 5 and10 μm remain in Peyer's patches, releasing protein for up to 1 month [18].

Oral administration of poly(D,L-lactic-*co*-glycolic) acid microspheres

PLGA microspheres have been proposed for oral immunotherapy for food allergies. The ease of oral administration could enhance patient compliance and comfort. Encapsulating antigen within PLGA microspheres can also help protect the entrapped antigen from degradation during transit through the gastrointestinal tract while allowing absorption of the bioactive antigen in an unaltered form [11,19]. Studies have shown that mice fed encapsulated protein had greater capacity to suppress specific serum IgG than soluble protein, but, if the encapsulated dose was too high, oral tolerance was broken and a strong, Th2 cell response was activated [20,21].

It is known that oral tolerance to allergenic foods can be induced using orally administered, soluble protein, but very high doses are required [22]. In 2000, Pecquet *et al.* [23] demonstrated that a single oral administration of beta-lactoglobulin (BLG)-loaded PLGA microspheres was able to induce oral tolerance in mice at a dose 10 000 times less than that required with soluble antigen. In 2002, Fattal *et al.* [24] also found that oral administration of BLG-loaded microspheres was able to decrease specific anti-BLG IgE at a dose 10 000 times less than that required with soluble antigen. In 2007, Gomez *et al.* [25] found that oral administration of ovalbumin-loaded microspheres was able to protect mice that had been sensitized to ovalbumin from experiencing anaphylactic shock when subsequently challenged with the antigen.

In 1997, a small, double-blind placebo-controlled human study was conducted by Litwin *et al.* [26], measuring serum antigen-specific IgG and IgE and nasal secretory IgA after oral administration of PLGA microspheres loaded with ragweed pollen extract. The study found that the treatment group had higher titers of antigen-specific IgG, a much lower seasonal increase in antigen-specific IgE and a small amount of antigen-specific IgA not present before immunization, as compared with controls. Symptom and medication scores were also lower in the treatment group as compared with controls. In another double-blind placebo-controlled human study in 2004, TePas *et al.* used a micro-encapsulated form of timothy grass pollen for oral immunotherapy. One daily treatment for 10 weeks caused a decrease in combined medication and symptom scores in 11/12 patients versus placebo along with decreased in-vitro lymphocyte proliferative response to antigen challenge in nine of 12. However, there were no significant changes noted in IgG, IgE or skin reactivity [27]. In 2005, Roth-Walter *et al.* [28] targeted birch pollen-loaded microspheres to murine intestinal M cells with the addition of alpha-L-fructose-specific *Aleuria aurantia* lectin. They found that oral administration induced antigen-specific IgG2a antibodies, but not IgG1 antibodies, suggesting that oral administration of these M cell-targeted microspheres may help shift the immune response toward a Th1 response.

Intranasal administration of poly(D,L-lactic-*co*-glycolic) acid microspheres

Intranasal therapy can be easily administered and may require lower antigen doses because the protein is not exposed to the low pH and digestive enzymes of the gastrointestinal tract. In 2008, Marazuela *et al.* [29] reported on the intranasal administration of PLGA microspheres loaded with Ole e 1. They reported that pretreatment of BALB/c mice with antigen-loaded microspheres led to a significant inhibition of serum allergen-specific IgE and IgG1, and an increase of specific IgG2 as compared with controls. IL-5 and IL-10 levels in splenocyte cultures were also suppressed in the pretreated mice. Further, histopathologic studies revealed that there was significantly less airway inflammation in the pretreated mice. This study suggested that intranasal immunization with Ole e 1-loaded microspheres was effective in preventing subsequent allergic sensitization to that antigen.

Subcutaneous administration of poly(D,L-lactic-*co*-glycolic) acid microspheres

It has been shown that the subcutaneous administration of protein-loaded microspheres produces a significantly higher immune response than oral administration, even if the protein dose is 10 times lower, and that subcutaneous exposure to allergenic protein as it is being released from microspheres does not lead to further sensitization to that protein [15,30]. In 2001, Iguarta *et al.* [10] demonstrated that subcutaneously injected microspheres loaded with Ole e 1 were able to invoke an immune response, as measured by an increase in antigen-specific IgM. A similar response was noted for the group receiving Ole e 1 suspended in saline, but this group received twice as many injections. In 2004, Scholl *et al.* [30] injected microspheres containing birch pollen antigen, Bet v 1, subcutaneously into previously sensitized mice. Levels of IgG1 decreased and levels of IgG2a increased along with IL-10, suggesting a downregulation of the Th2 response. However, antigen-specific IgE did not change in their study.

In 2007, Martinez Gomez *et al.* [31] demonstrated that antigen-specific immunotherapy, using subcutaneous injection of PLGA microspheres loaded with bee venom

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

PLGA2 was able to induce production of IgG2a in mice, and that higher titers of antibodies correlated with better protection against temperature drop when anaphylaxis was induced. In their 2009 study, Roman *et al.* [32••] determined that the addition of CpG motifs (Th1 immunoadjuvants) to ovalbumin-loaded PLGA microspheres did not have an effect on desensitization. Subcutaneous immunotherapy with antigen-loaded microspheres, regardless of presence of CpG motifs, elicited lower IgE/IgG2a ratios than those induced by allergen in solution. However, after the induction of anaphylaxis, only mice treated with antigen-loaded microspheres plus CpG were protected from death.

In 2009, Apicella *et al.* [33•] compared subcutaneous immunotherapy with ovalbumin-loaded PLGA microspheres, soluble ovalbumin and polymerized ovalbumin with respect to serum levels of antigen-specific IgE, IgG1 and IgG2a, the cutaneous anaphylaxis test, and IL-10, IFN-γ and IL-4 levels in splenocyte cultures after ovalbumin challenge. Results suggested that, among the different therapies tested, PLGA microspheres loaded with ovalbumin represented the most effective method of desensitization. Mice treated with microspheres had lower levels of IgG1 and higher levels of IgG2a, as well as an inhibitory effect on the cutaneous anaphylaxis test. IL-10 levels and IFN-γ levels were higher in mice treated with microspheres and soluble ovalbumin compared with polymerized ovalbumin.

In 2009, our group investigated whether subcutaneous microsphere immunotherapy (SMITH) could downregulate the antigen-specific IgE production in mice that had been sensitized to ovalbumin. One group received a low dose of ovalbumin-loaded microspheres, a second group received a high dose and the third group received blank microspheres. Although the antigen-specific IgE in the group receiving blank microspheres continued to rise after immunization, neither treatment group demonstrated a significant rise in antigen-specific IgE after that point (unpublished data).

Conclusion

Immunotherapy using PLGA microspheres loaded with allergenic proteins represents a potentially significant advancement in the treatment of allergic disease. Microsphere immunotherapy is able to deliver intact allergenic protein to specific target tissues of the immune system in a steady fashion for an extended period of time. Avoiding the bolus of allergenic protein seen with SCIT may not only reduce the risk of severe adverse reactions but also provide a more effective and convenient form of therapy. Current data demonstrate that microsphere immunotherapy promotes a more favorable, Th1-dominant immune pattern, while also providing a therapeutic benefit.

It is still unclear which route of delivery for microsphere immunotherapy is most appropriate under which circumstances, but the answers may open the door to new therapeutic options, such as the treatment of food allergies using a subcutaneous injection rather than oral immunotherapy. More information must also be known about the proper dosing of antigen-loaded microspheres to maximize both efficacy and safety, and strategies should be sought to improve encapsulation efficiency and extend the period of time in which proteins can be released. Current studies have examined microspheres loaded with only one allergenic protein, but microspheres loaded with multiple allergens need to be tested for use in polysensitized individuals. Looking toward the future, larger studies in the human population will be necessary to evaluate and compare the long-term therapeutic effects of microsphere immunotherapy against conventional subcutaneous and oral immunotherapy for airborne and food allergies.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 230).

1 Pang X, Chu CC. Synthesis, characterization and biodegradation of functio-
•• nalized amino acid-based poly(ester amide)s. Biomaterials 2010; 31:3745–3754.

This article provides a comprehensive review of the new and older polymer materials being used for drug delivery.

2 Tamber H, Johansen P, Merkle HP, *et al.* Formulation aspects of biodegradable polymeric microspheres for antigen delivery. Adv Drug Deliv Rev 2005; 57:357–376.

3 Sanders LM, Kent JS, McRae GI, *et al.* Controlled release of a luteinizing hormone-releasing hormone analogue from poly(D,L-lactide-co-glycolide) microspheres. J Pharm Sci 1984; 73:1294–1297.

4 Uhrich KE, Cannizzaro SM, Langer RS, *et al.* Polymeric systems for controlled drug release. Chem Rev 1999; 99:3181–3198.

5 Cohen EM, Ding H, Kessinger CW, *et al.* Polymeric micelle nanoparticles for
• photodynamic treatment of head and neck cancer cells. Otolaryngol Head Neck Surg 2010; 143:109–115.

This article describes a novel use for microspheres in the treatment of head and neck cancer using photodynamic therapy.

6 Burton MJ, Krouse JH, Rosenfeld RM. Extracts from The Cochrane Library: allergen injection immunotherapy for seasonal allergic rhinitis. Otolaryngol Head Neck Surg 2007; 136:511–514.

7 Bordignon V, Burastero SE. Multiple administrations of low-dose sublingual immunotherapy in allergic rhinoconjunctivitis. Ann Allergy Asthma Immunol 2006; 97:158–163.

8 Sharif S, Wheeler AW, O'Hagan DT. Investigation of the structural and immunologic integrity of an allergen entrapped in poly(lactide-co-glycolide) microparticles. In: 21st International symposium on controlled release of bioactive materials. Nice, France: Controlled Release Society; 1994. pp. 292–295.

9 Sharif S, Wheeler AW, O'Hagan DT. Biodegradable microparticles as a delivery system for the allergens of *Dermatophagoides pteronyssinus* (house dust mite): preparation and characterization of microparticles. Int J Pharm 1995; 119:239–246.

10 Iguarta M, Hernandez RM, Gutierro I, *et al.* Preliminary assessment of the immune response to *Olea europaea* pollen extracts encapsulated into PLGA microspheres. Pharm Develop Technol 2001; 6:621–627.

11 Guerin V, Dubarry M, Robic D, *et al.* Microsphere entrapped bee-venom phospholipase A2 retains specific IgE binding capacity: a possible use for oral specific immunotherapy. J Microencapsulat 2002; 19:761–765.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

12 Batanero E, Barral P, Villalba M, *et al.* Encapsulation of Ole e 1 in biodegradable microparticles induced Th1 response in mice: a potential vaccine for allergy. J Control Release 2003; 92:395–398.

13 Sinha VR, Trehan A. Biodegradable microspheres for protein delivery. J Control Release 2003; 90:261–280.

14 Hora MS, Rana RK, Nunberg JH, *et al.* Release of human serum albumin from poly(lactide-co-glycolide) microspheres. Pharm Res 1990; 7:1190–1194.

15 Igartua M, Hernandez RM, Esquisabel A. Enhanced immune response after subcutaneous and oral immunization with biodegradable PLGA microspheres. J Control Release 1998; 56:63–73.

16 Peyre M, Fleck R, Hockley D, *et al.* In vivo uptake of an experimental microencapsulated diphtheria vaccine following sub-cutaneous immunization. Vaccine 2004; 22:2430–2437.

17 Tabata Y, Ikada Y. Macrophage phagocytosis of biodegradable microspheres composed of L-lactic acid/glycolic acid homo- and copolymers. J Biomed Mater Res 1988; 22:837–858.

18 Eldridge JH, Staas JK, Chen D, *et al.* New advances in vaccine delivery systems. Semin Hematol 1993; 30 (Suppl 4):16–24 (discussion 25).

19 Scholl I, Boltz-Nitulescu G, Jensen-Jarolim E. Review of novel particulate antigen delivery systems with special focus on treatment of type I allergy. J Control Release 2005; 104:1–27.

20 Elson CO, Tomasi M, Dertzbaugh MT, *et al.* Oral-antigen delivery by way of a multiple emulsion system enhances oral tolerance. Ann N Y Acad Sci 1996; 778:156–162.

21 Flanagan M, Jain S, Michael JG. Breaking of oral tolerance by encapsulated antigen. Ann N Y Acad Sci 1996; 778:373–375.

22 Pecquet S, Pfeifer A, Gauldie S, Fritsche R. Immunoglobulin E suppression and cytokine modulation in mice orally tolerized to beta-lactoglobulin. Immunology 1999; 96:278–285.

23 Pecquet S, Leo E, Fritsche R, *et al.* Oral tolerance elicited in mice by beta-lactoglobulin entrapped in biodegradable microspheres. Vaccine 2000; 18:1196–1202.

24 Fattal E, Pecquet S, Couvreur P, Andremont A. Biodegradable microparticles for the mucosal delivery of antibacterial and dietary antigens. Int J Pharm 2002; 242:15–24.

25 Gomez S, Gamazo C, Roman BS, *et al.* Gantrez AN nanoparticles as an adjuvant for oral immunotherapy with antigens. Vaccine 2007; 25:5263–5271.

26 Litwin A, Flanagan M, Entis G, *et al.* Oral immunotherapy with short ragweed extract in a novel encapsulated preparation: a double-blind study. J Allergy Clin Immunol 1997; 100:30–38.

27 TePas EC, Hoyte EG, McIntire JJ, *et al.* Clinical efficacy of microencapsulated timothy grass pollen extract in grass-allergic individuals. Ann Allergy Asthma Immunol 2004; 92:25–31.

28 Roth-Walter F, Scholl I, Untersmayr E, *et al.* Mucosal targeting of allergen-loaded microspheres by *Aleuria aurantia* lectin. Vaccine 2005; 25:2703–2710.

29 Marazuela EG, Prado N, Moro E, *et al.* Intranasal vaccination with poly(lactide-co-glycolide) microparticles containing a peptide T of Ole e 1 prevents mice against sensitization. Clin Exp Allergy 2008; 38:520–528.

30 Scholl I, Weissenbock A, Forster-Waldl E, *et al.* Allergen-loaded biodegradable poly(D,L-lactic-co-glycolic) acid nanoparticles down-regulate an ongoing Th2 response in the BALB/c mouse model. Clin Exp Allergy 2004; 34:315–321.

31 Martinez Gomez JM, Fischer S, Csaba N, *et al.* A protective allergy vaccine based on CpG- and protamine-containing PLGA microparticles. Pharm Res 2007; 24:1927–1934.

32 •• Roman BS, Irache JM, Gomez S, *et al.* Co-delivery of ovalbumin and CpG Motifs into microparticles protected sensitized mice from anaphylaxis. Int Arch Allergy Immunol 2009; 149:111–118.
The authors of this article loaded the immunoadjuvant CpG along with ovalbumin into microspheres and demonstrated a therapeutic benefit for mice when anaphylaxis was induced.

33 • Apicella C, Roldan ER, Chiapetta DA, *et al.* Asymmetric IgG antibodies induced by different immunotherapies in a murine model of allergy. Immunol Invest 2009; 38:572–588.
The authors compared several preparations of ovalbumin and determined that PLGA–ovalbumin produced the greatest shift from a Th2 to a Th1 immune pattern.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.