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# Directed cell migration via chemoattractants released from degradable microspheres

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**Abstract**

Chemotaxis, cell migration directed by spatial concentration gradients of chemoattractant molecules, is critical for proper function of the immune system. Materials capable of generating defined chemoattractant gradients via controlled release may be useful for the design of improved vaccines and immunotherapies that draw specific cells to an immunization site. To this end, we encapsulated formyl-Nle-Leu-Phe-Nle-Tyr-Lys (fN'LFN'YK) peptides or macrophage inflammatory protein-3α (MIP-3α or CCL20) in degradable poly(lactide-co-glycolide) microspheres that provided sustained release for more than 2 weeks in vitro. fN'LFN'YK and MIP-3α chemoattract dendritic cells (DCs), the key antigen-presenting cells involved in generation of primary immune responses, and their precursors, monocytes. Using an in vitro videomicroscopy migration assay, we detected strong chemotaxis of human monocytes and monocyte-derived DCs through 3D collagen gels toward microspheres releasing fN'LFN'YK. Similarly, microparticles releasing MIP-3α were able to attract mouse bone marrow-derived dendritic cells. Strikingly, prolonged attraction of DCs from distances up to 500 μm from the source to the point of contact with individual microspheres was observed. Such microspheres could be of general interest for the design of vaccines that promote adaptive immunity and as a platform for studying the biology of chemotaxis in vitro and in vivo.


*Keywords:* Chemotaxis; Controlled; Drug release; Microspheres; Immune response

## 1. Introduction

Cells of the immune system exhibit complex patterns of trafficking among the primary and secondary lymphoid organs and the peripheral tissues as part of homeostasis and immune surveillance [1–3]. Spatial and temporal orchestration of lymphocyte migration is achieved by a diverse family of chemokines, typically ~10 kDa polypeptides which bind related families of G protein-coupled receptors [4–6]. Chemokines and other chemoattractant molecules are produced at local sites and diffuse to form soluble or solid-phase concentration gradients; cells expressing the appropriate chemokine receptors typically migrate up chemoattractant gradients toward their source. Chemokines and cognate receptors are now known, which guide naive T and B cells to their local niches within the secondary lymphoid organs [7,8]; attract antigen-presenting cells (APCs), effector and memory T and B cells, and innate immune cells to sites of infection [9]; and guide antigen-loaded APCs to lymph nodes [10].

The sensitivity of immune cells to specific chemokines might be exploited in the design of novel immunotherapies and vaccines, by inducing specific cell types to concentrate at a local depot of antigen. For example, primary immune responses are initiated when professional APCs known as

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dendritic cells (DCs) engulf foreign antigens and become activated, migrate to draining lymph nodes, and present these captured antigens to T cells to initiate T-cell activation [11–13]. In the presence of a pathogen, stromal and inflammatory cells in the periphery initiate this cascade by secreting chemokines that selectively attract immature (unactivated) DCs and their precursors (e.g., monocytes) to the sites of infection or inflammation [13,14]. A number of chemoattractants are known to elicit directed migration of DCs and monocytes in this context, including monocyte chemotactic protein-1 (MCP-1), MCP-2, MCP-3, macrophage inflammatory protein-1$\alpha$ (MIP-1$\alpha$), MIP-1$\beta$, Regulated upon Activation Normal T cell Expressed and Secreted (RANTES), C5a, $\beta$-defensins, and bacterially derived formyl peptides [15–20]. Because the frequency of DCs in blood and peripheral tissues is low (typically ~1% of cells or fewer [21,22]), vaccines that mimic DC recruitment to inflamed tissues by creating a local chemoattractant source may significantly enhance immune responses. Attraction of resting 'immature' DCs to an immunization site could increase the number of DCs loaded with antigen, and subsequently, the number of naive T cells activated in the draining lymph nodes.

Several prior studies have sought to develop vaccines that apply this principle, by immunizing with DNA plasmids that encode an antigen of interest as well as a chemoattractant molecule [23–29]. This approach has been shown to trigger enhanced DC infiltrates at injection sites and improved protection against tumors and model pathogens. However, DNA immunization provides little control over the amount of chemokine produced or the time course of its expression, which limits the ability of this approach to control the magnitude and duration of DC attraction. These characteristics also make DNA immunization an intractable method for dissecting the biology of chemokine functions in immune cell trafficking.

As an alternative, Kumamoto et al. [30] demonstrated that implanted poly(ethylene-co-vinyl acetate) rods releasing a model protein antigen and MIP-3$\beta$ (a chemoattractant for activated 'mature' DCs) drew DCs to the implant site and resulted in enhanced protection against E.G7 and 3LL tumors in mice. This approach allowed a known amount of chemokine to be implanted with defined release kinetics. However, the use of non-degradable polymer rods that require implantation and eventual retrieval is not attractive for a practical vaccine. In addition, EVAc releases protein rapidly, over a course of ~24 h due to rapid swelling of the matrix with water; such a system cannot deliver chemokine over longer time periods which may be optimal for maximization of the immune response [27].

Given these promising prior results, we sought to develop a system which could serve both as a potential platform for manipulating lymphocyte trafficking in immunotherapies and as a basic tool for quantitatively studying the role of chemokines in controlling immune cell trafficking in vitro and in vivo. To this end, we examined controlled release microspheres as an injectable formulation that could mimic the generation of chemoattractant gradients generated in situ in natural acute infections and enrich professional APCs at an immunization site. We first carried out simple transport modeling to compare the concentration gradients expected near a depot of controlled release microspheres to that predicted for a bolus injection of freely diffusing chemokine. To examine this strategy experimentally, we fabricated degradable poly(lactide-co-glycolide) microspheres as an injectable formulation that could provide controlled release of DC-specific chemokines. Two chemoattractants with different physical properties were chosen for these studies: a hydrophobic formyl peptide variant formyl-Nle-Leu-Phe-Nle-Try-Lys (fN'LFN'YK), representative of pathogen-derived chemoattractants present during bacterial infections [31] and the 7.9 kDa C-C chemokine MIP-3$\alpha$ (a.k.a. CCL20) [32–34]. These chemokines attract immature DCs and their monocyte precursors, via binding to the formyl peptide receptor and CCR6, respectively. To assay the ability of controlled release microspheres to guide migration of DCs and monocytes to a local site, we developed an assay for chemotaxis based on time-lapse videomicroscopy observation of live cell migration through three dimensional (3D) collagen gels in vitro, which is related to direct observation assays used in prior cell migration studies of immune cells [35–37] and other cell types [38]. Chemoattractants released from PLGA microspheres were found to elicit strong, sustained attraction of human DCs and monocytes as well as murine DCs for a least 8 h. Slow release of chemokine from microspheres was found to provide significantly stronger attraction compared to bolus delivery of freely diffusing attractant. These microspheres allow defined total amounts of chemokine to be delivered to known sites with known release kinetics, making them of interest both for the design of improved vaccines and as tools for dissecting the role of chemoattractants in immunobiology. In addition, control of cellular chemotaxis via chemoattractant-releasing biomaterials could be a powerful strategy for tissue engineering, (e.g., for guided angiogenesis), where guided physical organization of multiple cell types according to wound healing or developmental principles is of interest.

## 2. Materials and methods

### 2.1. Modeling of chemoattractant gradients generated by controlled release

Theoretical concentration profiles of MIP-3$\alpha$ diffusing through a collagen gel were calculated via a finite element model using Matlab software (MathWorks;