4f Polymeric Biomaterials with Tailored Microstructures, Nanostructures, and Bioactive Surface Chemistries for Drug Delivery and Tissue Engineering

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Introduction

My research involves biomedical applications of chemical engineering, by applying polymer science to relevant problems in drug delivery and tissue engineering.

I earned my Ph.D. from Iowa State University, where my work resulted in the development of a single-dose vaccine delivery vehicle based on bioerodible polymer microspheres with tailored microstructures and nanostructures. This work involved:

- Experimental and theoretical investigation of the polymer physics of the particular polymer system in order to determine its microstructure and nanostructure.
- Mathematical modeling of the erosion and drug release kinetics.
- Studies to investigate the vaccine efficacy and capability to modulate the immune response mechanism.

This work resulted in the publication of twelve peer-reviewed journal articles and one patent.

Currently, I have a postdoctoral fellowship award from the National Research Council with a joint appointment at the National Institutes of Health and the National Institute of Standards and Technology. I am developing surfaces and scaffolds that present covalently attached bioactive chemistries that promote the migration of specific cell types important to tissue engineering. By promoting migration, these materials could overcome challenges associated with cell seeding on three-dimensional scaffolds and enhance wound healing. Additionally, I am demonstrating how these materials can aid cellular and developmental biologists in studying phenomena that contribute to cell migration. In addition to tissue engineering, this work could have impacts in areas such as embryonic development and tumor metastasis, for which cell migration is a critical phenomenon. *I'll be presenting this work at talk 594f on Friday, November 4th at 1:34 PM in Regency Ballroom G.*

These two projects are briefly outlined below:

Single-dose vaccine based on bioerodible microspheres with tailored microstructures and nanostructures

In 2003, the National Institutes of Health, the World Health Organization, and the Bill and Melinda Gates Foundation identified the development of single-dose vaccines as number one on the list of Grand Challenge in Global Health (http://www.grandchallengesgh.org/). This work describes the investigation of bioerodible polyanhydrides as controlled drug and vaccine delivery vehicles, and the development of a single-dose vaccine carrier based on these materials. The polymers studied are based on the 1,6-bis(*p*-carboxyphenoxy)hexane (CPH) and sebacic acid (SA) monomers. These two materials erode at vastly different rates and can be combined in random copolymers or blends to achieve tailored erosion kinetics. The hydrophobic nature of these materials offers the potential to stabilize proteins, and their mutual incompatibility and semicrystallinity provide an interesting phase behavior, which can be exploited to aid in tailoring the release kinetics. This work involved the theoretical and experimental description of the microstructure and nanostructure of polyanhydride copolymers, the development of an injectable

drug delivery vehicle based on polyanhydride microspheres, and the development of accurate kinetic models of polymer erosion that provide details of the erosion phenomenon that are difficult to obtain experimentally, but may impact the stability of therapeutic proteins.

Description of the microstructure and nanostructure of polyanhydride copolymers

Through a combination of small angle X-ray scattering, atomic force microscopy, solid-state NMR, optical microscopy, and molecular simulations, detailed descriptions of the copolymer and blend microstructures and nanostructures are obtained. This microstructure/nanostructure includes microphase separation in the amorphous phase for the copolymers, crystalline/amorphous phase separation for the copolymers and homopolymers, and a phase diagram for the hompolymer blends. This comprehensive description of the microstructural details is essential to understanding the complex erosion and drug release kinetics exhibited by these materials.

Development of an injectable drug delivery system based on polyanhydride microspheres

Release kinetics experiments are performed *in vitro* and *in vivo* to ascertain the affects of microstructural and nanostructural characteristics and to study the immune responses to a model antigen, tetanus toxoid (TT). Tailored release profiles of small molecular weight model drugs are demonstrated by combining microspheres with different erosion kinetics in "cocktails." Several vaccine formulations are investigated to determine which combinations of polymer hydrophobicity, protein stability, and protein release kinetics offer the greatest potential to achieve protective immunity in a single dose. A single-dose vaccine formulation that induces a secondary immune response characterized by sustained high titers of high avidity antibody is demonstrated in a mouse model. Additionally, it is shown that the *in vivo* immune response mechanism can be tuned by altering the vaccine formulation. The ability to alter the immune response mechanism without the addition of noxious adjuvants is a unique and valuable feature of this delivery system.

Accurate erosion and drug release kinetics models

The models incorporate the details of the polymer microstructure and provide molecular level descriptions of the complex process of erosion. Important phenomena that occur during erosion, such as porosity change, crystallinity change, monomer accumulation, and pH change in the eroding zone are described. Although these phenomena are difficult to accurately measure experimentally, they can be predicted by accurate erosion models such as the one presented. Understanding these phenomena is essential to the rational design of controlled release systems for macromolecular drugs (e.g. proteins, vaccines).

Bioactive peptide gradients for promotion and assay of cell migration

The migration of cells that are involved in the early (inflammation) stage of wound healing, such as neutrophils, macrophages, and T lymphocytes has been extensively studied. However, the migration of slower migrating cells, such as fibroblasts and endothelial cells, which are involved in the later stages (proliferation and remodeling) of wound healing, is more difficult to study. Techniques such as Boyden chamber assays, in which the cells are allowed to migrate across a membrane or filter may be good models for processes such as metastasis or extravasation, but may not be relevant models for connective tissue cells migrating across a wound. Cells migrate to a wound in response to concentration gradients of soluble chemotactic factors by a process known as chemotaxis. Because these gradients are inherently unstable, it is difficult to use them to study the migration of slower moving endothelial cells and

fibroblasts. Endothelial cells and fibroblasts can also respond to bound (rather than soluble) peptide gradients in a process known as haptotaxis. This process may be exploited in novel tissue engineering scaffolds to recruit cells to a wound site and promote the migration of cells into a tissue engineering construct. A technique for preparing surfaces and three-dimensional scaffolds with covalently bound peptide gradients is discussed. Bioactive peptides from Laminin-1, a basement membrane protein, that are known to promote adhesion or migration of endothelial cells or fibroblasts are used to develop surfaces and three-dimensional scaffolds with covalent peptide gradients. Time-lapse video microscopy of cell culture is used to monitor the behavior of cells with respect to the gradients. This approach offers a new technique for screening the haptotactic potential of peptides. It also permits the study of haptotaxis for slowly migrating cells that are difficult to characterize by other techniques. Finally, these materials are readily adaptable to clinical applications of tissue engineering as they do not contain unstable gradients and are based on materials with well-established biocompatibility for a variety of in vivo applications.

Journal Publications

1. A.S. Determan, J. Wilson, **M.J. Kipper**, M. Wannemuehler, and B. Narasimhan, "Protein Stability in the Presence of Polymer Degradation Products: Consequences for Controlled Release Formulations." *Biomaterials*, (Submitted, 2005).

2. **M.J. Kipper**, J. Wilson, M. Wannemuehler, and B. Narasimhan, "Single dose Tetanus Vaccine based on Bioerodible Polyanhydride Microspheres can Modulate Immune Response Mechanism." *J. Biomed. Mater. Res. A.*, (In press, 2005).

3. **M.J. Kipper**, S. Seifert, S.-S. Hou, K. Schmidt-Rohr, P. Thiyagarajan, and B. Narasimhan, "Nanoscale Morphology of Polyanhydride Copolymers." *Macromolecules*, **38**, 8468-8472, 2005.

4. **M.J. Kipper**, S. Seifert, P. Thiyagarajan, and B. Narasimhan, "Small-Angle X-Ray Scattering to Discern Microstructure of Semicrystalline Polyanhydrides for Drug Delivery." *Adv. X-Ray Anal.,* **48**, 73-81, 2005.

5. **M.J. Kipper,** and B. Narasimhan, "A Molecular Description of Erosion Phenomena in Biodegradable Polymers." *Macromolecules*, **38**, 1989-1999, 2005.

6. **M.J. Kipper,** S. Seifert, P. Thiyagarajan, and B. Narasimhan, "Morphology of Polyanhydride Copolymers: Time Resolved SAXS Studies of Polyanhydride Crystallization." *J. Polym. Sci., Part B: Polym. Phys.*, **43**, 463-477, 2005.

7. **M.J. Kipper,** S. Seifert, P. Thiyagarajan, and B. Narasimhan, "Understanding Polyanhydride Blend Phase Behavior Using Scattering, Microscopy, and Molecular Simulations." *Polymer*, **45**, 3329-3340, 2004.

8. B. Narasimhan and **M.J. Kipper**, "Surface-Erodible Biomaterials for Drug Delivery." *Adv. Chem. Engr.,* **29**, 169-218, 2004.

9. C. Berkland, **M.J. Kipper,** K. Kim, B. Narasimhan, and D.W. Pack, "Microsphere Size, Precipitation Kinetics and Drug Distribution Control Drug Release from Biodegradable Polyanhydride Microspheres." *J. Controlled Release*, **94**, 129-141, 2004.

10. **M. J. Kipper**, E. Shen, A. Determan, and B. Narasimhan, "Design of an Injectable System Based on Bioerodible Polyanhydride Microspheres for Sustained Drug Delivery." *Biomaterials*, **23**, 4405-4412, 2002.

11. D. Larobina, **M.J. Kipper**, G. Mensitieri, and B. Narasimhan, "Mechanistic Understanding of Degradation in Bioerodible Polymers for Drug Delivery." *AIChE J.*, **48**, 1260-1270, 2002.

12. E. Shen, **M.J. Kipper**, B. Dziadul, M.-K. Lim, and B. Narasimhan, "Mechanistic Relationships Between Polymer Microstructure and Drug Release Kinetics in Bioerodible Polyanhydrides." *J. Controlled Release*, **82**, 115-125, 2002.

Patent

M.J. Kipper, B. Narasimhan, J.H. Wilson, and M.J. Wannemuehler, Single-Dose Controlled-Release Vaccine Formulations Based on Polyanhydride Microspheres for Control of Immune Response Mechanism, U.S. patent application number 60/623,711, October 29, 2004.