In Vitro and Histological Analysis of Polymer Microsphere Delivery

Jonathan Yakubov • George Zhou • Vérida Léandre • Christopher Baker • Seeta Rajpara • Edith Mathiowitz

Department of Molecular Pharmacology, Physiology and Biotechnology. Brown University, Providence, Rhode Island

INTRODUCTION

Cellular recruitment has diverse tissue engineering applications, ranging from wound healing[1] to the treatment of metabolic disorders.[2] Chemoattractants encapsulated in hydrogels, microspheres, scaffolds, and other systems have been studied as recruitment agents for a variety of these applications.[1,3,4] Thus the goal of our project is to study how microspheres can be used to recruit progenitor cells. To achieve this, we histologically analyzed the presence of non-degradable polystyrene (PS) microspheres in rats delivered via either oral gavage or subcutaneous injection. These spheres serve as a model for bioresorbable polylactic acid (PLA) microspheres. Next, we examined the effects that various solvents used during the histology process have on polymers, as their use can cause artefacts in histology slides. Lastly, we assayed cell response to dexamethsone, a drug involved in adipogenic differention[3]. This work furthers our understanding of cell recruitment through microspheres and may aid in further development of recruitment based therapies.

SOLVENT EFFECTS

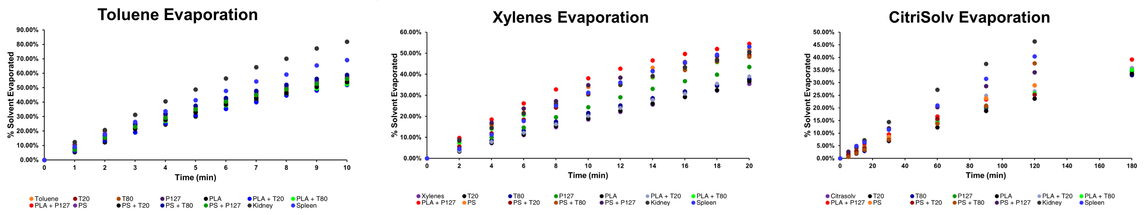
Methods

Solvent Evaporation

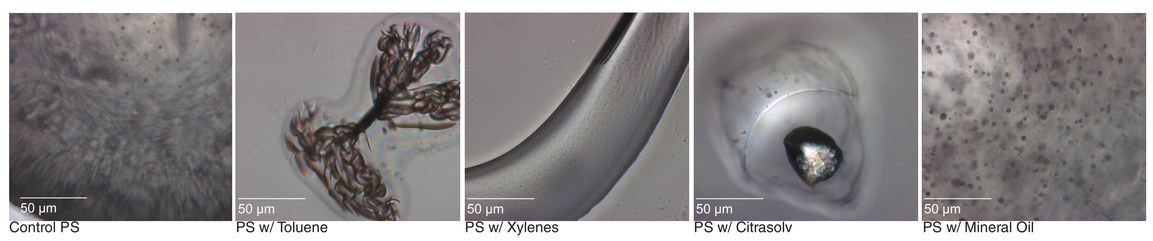
75 µL of various solvents were pipetted into an O-ring placed on a glass microscope slide and mass loss from evaporation was recorded on a scale. This was repeated with either unprocessed PLA and PS or spleen and kidney tissue sections placed in the center of the O-ring. Optical microscopy was performed for qualitative analysis.

Results

- Solvent evaporation rates are mostly linear. Surfactants caused no significant changes in evaporation rate. Differing experimental conditions caused variations in evaporation.

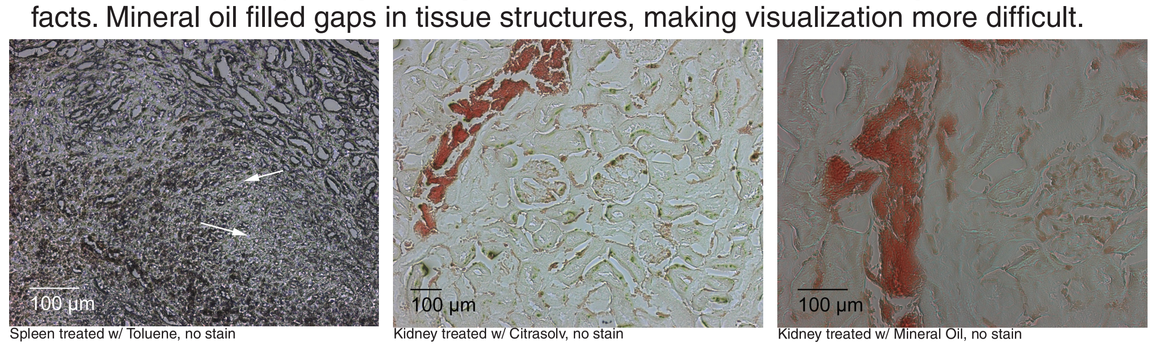


- Toluene and Xylenes dissolved PS. CitriSolv caused a yellow-green discoloration.



Control PS | PS w/ Toluene | PS w/ Xylenes | PS w/ Citrasolv | PS w/ Mineral Oil

- Toluene caused polymer gumming (arrows). CitriSolv caused yellow-green color artefacts. Mineral oil filled gaps in tissue structures, making visualization more difficult.

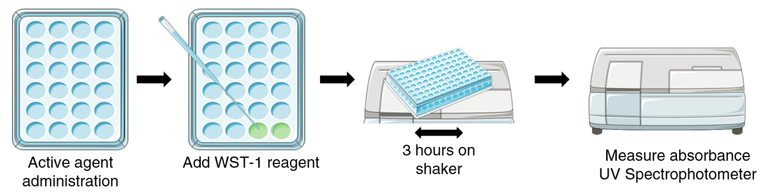


Spleen treated w/ Toluene, no stain | Kidney treated w/ Citrasolv, no stain | Kidney treated w/ Mineral Oil, no stain

CELL PROLIFERATION

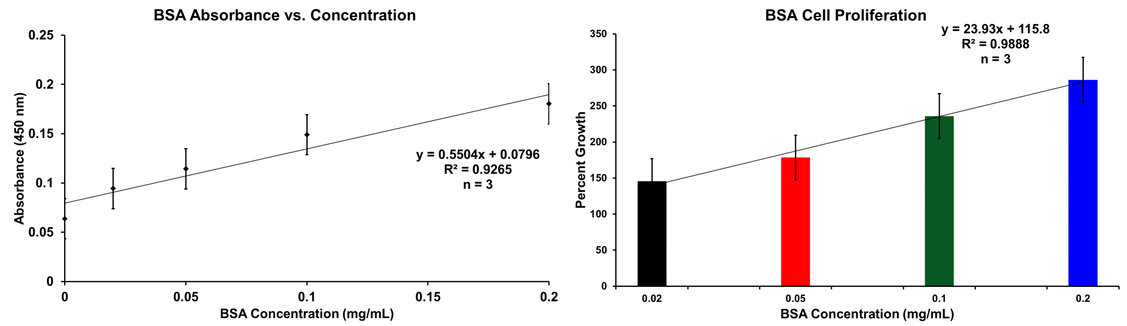
Methods

WST-1 Proliferation Assay

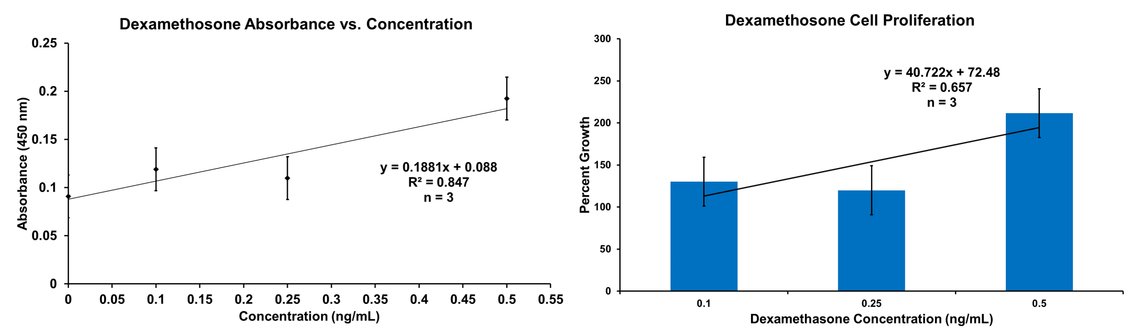


Results

- Bovine serum albumin (BSA) was used as a model for this bioactivity assay.
- Graphs show positive correlation between BSA and cellular proliferation of NIH 3T3 fibroblast cells.



- Physiologically relevant concentrations of dexamethasone demonstrated ability to cause cellular proliferation.
- Percent growth was highest when 0.5 ng/mL of drug was delivered.



HISTOLOGY

Methods

H&E Staining

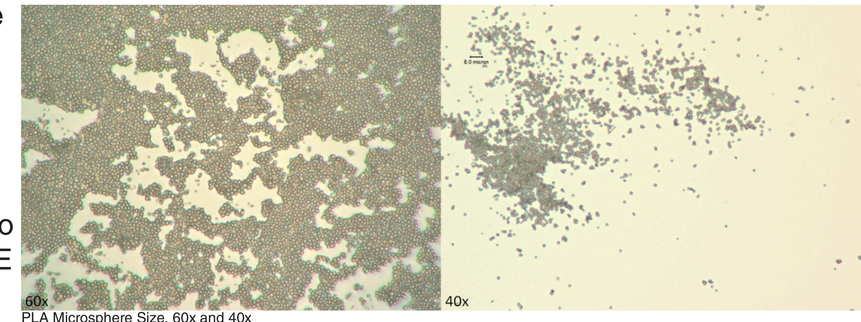
Slides were prepared, then dyed in Hemotoxylin and Eosin to examine cellular structure and nuclei.

Oil Red O Staining

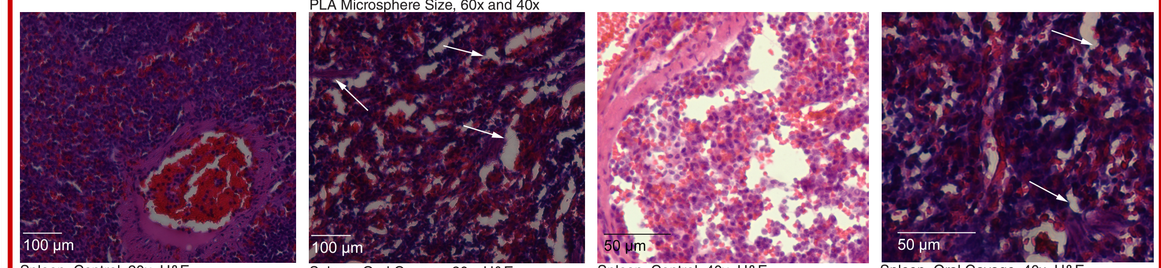
Slides were prepared, then dyed in Oil Red O with Hematoxylin counterstain to examine lipid distribution

Results

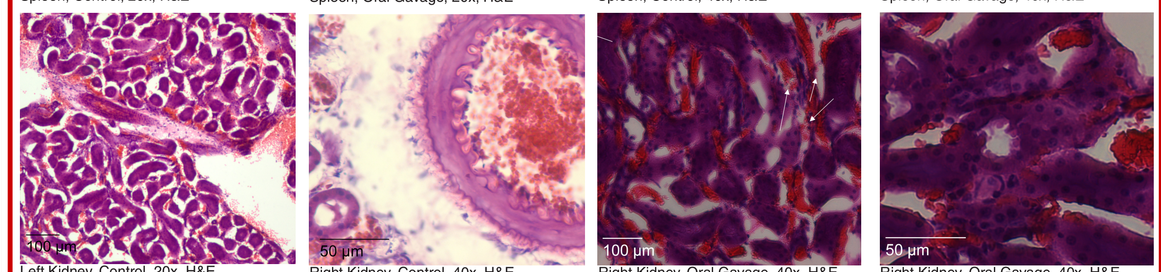
- Microspheres were successfully delivered via both oral and subcutaneous routes
- Microspheres are small and difficult to observe under H&E staining.



PLA Microsphere Size, 60x and 40x

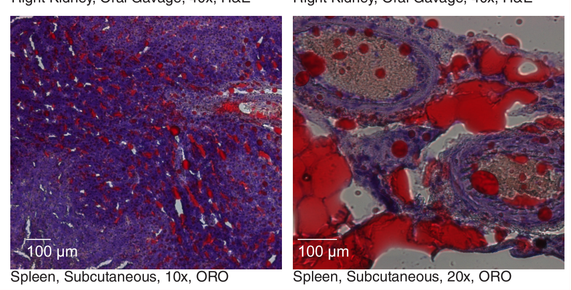


Spleen, Control, 20x, H&E | Spleen, Oral Gavage, 20x, H&E | Spleen, Control, 40x, H&E | Spleen, Oral Gavage, 40x, H&E



Left Kidney, Control, 20x, H&E | Right Kidney, Control, 40x, H&E | Right Kidney, Oral Gavage, 40x, H&E | Right Kidney, Oral Gavage, 40x, H&E

- Arrows point to regions containing microspheres in H&E images above.
- Oil Red O images to right show lipid content of spleen and kidney in red.
- Lipid content affects ability to process tissue.



Spleen, Subcutaneous, 10x, ORO | Spleen, Subcutaneous, 20x, ORO

DISCUSSION

Solvent Effects

- Evaporation rates not greatly affected by surfactants.
- Toluene and Xylene both dissolved unprocessed PS.
- Toluene processing of tissue caused polymer gumming.
- CitriSolv resulted in yellow-green coloration of tissue, which can disrupt histological analysis and stains.
- Mineral oil makes histological visualization more difficult.
- Future Direction: Better control of environmental factors during evaporation experiments.

Cell Proliferation

- WST-1 assay suggests that BSA has stimulatory capacity for NIH 3T3 fibroblast cells.
- Strong correlation between BSA and cell proliferation
- Dexamethasone also shows stimulatory capacity for NIH 3T3 fibroblast cells.
- Strong correlation between drug and cell proliferation
- Future Direction: More experiments to make data more conclusive

Histology

- Microspheres were observed in both kidney and spleen tissue sections.
- Both oral gavage and subcutaneous injection demonstrate effective delivery of microspheres
- Delivery of microspheres will be important in releasing proteins that recruit progenitor cells.

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