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Spatiotemporal effects of a controlled-release anti-inflammatory drug on the cellular dynamics of host response

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ABSTRACT

In general, biomaterials induce a non-specific host response when implanted in the body. This reaction has the potential to interfere with the function of the implanted materials. One method for controlling the host response is through local, controlled-release of anti-inflammatory agents. Herein, we investigate the spatial and temporal effects of an anti-inflammatory drug on the cellular dynamics of the innate immune response to subcutaneously implanted poly(lactic-co-glycolic) microparticles. Noninvasive fluorescence imaging was used to investigate the influence of dexamethasone drug loading and release kinetics on the local and systemic inhibition of inflammatory cellular activities. Temporal monitoring of host response showed that inhibition of inflammatory proteases in the early phase was correlated with decreased cellular infiltration in the later phase of the foreign body response. We believe that using controlled-release anti-inflammatory platforms to modulate early cellular dynamics will be useful in reducing the foreign body response to implanted biomaterials and medical devices.

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1. Introduction

One major challenge to clinical application of biomaterials and medical devices is their potential to induce a non-specific host response [1–8]. This reaction involves the recruitment of early innate immune cells such as neutrophils and macrophages, followed by fibroblasts which deposit collagen to form a fibrous capsule surrounding the implanted object [1,8–11]. Fibrotic cell layers can hinder electrical [12] or chemical communications and prevent transport of analytes [13–15] and nutrients, thus leading to the eventual failure of many implantable medical devices such as glucose sensors [3,4,16], neural probes [17], immunoisolated pancreatic islets [18–20] and biodegradable polymeric stents [5].

The incorporation of controlled-release delivery systems of anti-inflammatory drugs into medical devices has been proposed to mitigate host response and improve device durability [21–25]. This approach has shown promise in a number of clinical applications. For example, controlled elution of steroids from pace-maker leads has reduced fibrosis formation and enhanced long-term electrical communication between the leads and surrounding cardiac tissue [12]. However, similar attempts to improve the performance of other medical devices such as implanted glucose sensors [26] and immunoisolated islets for diabetes therapy have proven challenging [1]. There remains a substantial need to better understand the immunomodulatory effects of anti-inflammatory drugs on the host-tissue biology at the implant site [21]. Such knowledge can lead to better design of controlled-release drug delivery systems to improve the biocompatibility of implanted medical devices.

Researchers developing controlled-release drug formulations to mitigate host response have largely focused on decreasing the number of inflammatory cells infiltrating the host-device interface. Hickey et al. designed a mixed microsphere system containing dexamethasone, a steroidal anti-inflammatory drug, to achieve zero-order *in vitro* release kinetics and suppress tissue response to thread-induced injuries in rats for up to one month [27,28]. Recent studies on a hydrogel composite containing dexamethasone-loaded poly(lactic-co-glycolic) (PLGA) particles also suggested that sustained release of this drug may minimize the inflammatory reactions at the tissue-material interface [29–31]. While these studies have provided invaluable information, they only addressed the effects of these drug delivery systems via *ex vivo* analysis of the

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cell types, quantity and distribution in excised tissues. However, various factors in the design of controlled-release formulations such as drug selection, drug loading, particle sizes and corresponding release kinetics can dynamically affect a range of biological activities in the host response. The presence of anti-inflammatory drugs may alter not only the quantity and variety of immune cells recruited but also the kinetics of cellular activities such as the secretion of inflammatory enzymes or cell signaling pathways [32,33]. *In vivo* cellular secretory products might affect the degradation rate of the polymeric matrix [34–36] used to encapsulate drugs, and are partly responsible for the discrepancy between *in vitro* and *in vivo* release kinetics [37]. Therefore, we hypothesized that monitoring the spatial and temporal dynamics of enzymatic activity in the host response will offer new insight into the efficacy of controlled-release systems of anti-inflammatory drugs.

In this study, we examined the real-time effects of controlled-release anti-inflammatory therapeutics on the host response to subcutaneously implanted polymeric materials. Poly(lactic-co-glycolic) (PLGA 50/50) microparticles with and without dexamethasone were subcutaneously injected in a six spot array on the dorsal side of immunocompetent mice. Monitoring the *in vivo* activity of cathepsins, a class of inflammatory proteases, by noninvasive fluorescent imaging revealed that microparticles with low drug loading (1.3 wt%) locally inhibited these enzymes, while high drug loading (26 wt%) formulation resulted in systemic immunosuppression. The low dexamethasone loading (1.3 wt%) was sufficient to attenuate the coverage of the implanted polymer by fibrotic cell layers. Temporal monitoring of the anti-inflammatory effect was carried out by *in vivo* imaging and *ex vivo* histological analysis.

2. Materials and methods

2.1. Fabrication and characterization of PLGA microparticles

Microparticles with or without dexamethasone were prepared using a single-emulsion method [38] with biodegradable PLGA 50/50 (inherent viscosity of 0.95–1.20 dl/g) from Lactel (Pelham, AL). Typically, a 5 mL solution of PLGA and dexamethasone dissolved in dichloromethane, at concentrations of 40 mg/ml and 2 mg/ml respectively, was quickly added to a 25 mL solution of 1% (w/v) polyvinyl alcohol and homogenized for 60 s at 5000 rpm (Silverson L4R, Silverson Machines Ltd., Cheshire, England). The resulting suspension was quickly decanted into 75 mL of deionized water and stirred for 30 s prior to rotary evaporation (Buchi Rotavap, Buchi, Switzerland) for 3 min. The suspension was washed five times by centrifugation at 3000 rpm for 3 min. The particles were collected by filtration using 0.2 μm filter, flash-frozen in liquid nitrogen, and lyophilized to dryness. Particle size distribution and morphology were examined by Scanning Electron Microscopy (JSM-6060, Jeol Ltd., Peabody, MA, USA). Fluorescence spectra of the PLGA polymer microparticles were collected by a Fluorolog-3 spectroflurometer (Horiba Yvon Jobin, Edison, NJ, USA). The dexamethasone loading of all microparticles was determined by dissolving 2 mg of microspheres in 1 mL of acetonitrile and comparing the resulting UV absorbance at 234 nm to a standard curve of known concentrations of dexamethasone in acetonitrile.

2.2. In vitro drug release kinetics

The sample preparation and separation methods reported elsewhere were utilized to study the release of drug from microparticles [39]. Briefly, 3.5 mg of dexamethasone-loaded PLGA microparticles were suspended in 1 mL of 0.9% (w/v) NaCl solution in a 1.5 mL centrifuge tube. The centrifuge tube was incubated at 37 °C on a tilt-table (Ames Aliquot Mixer, Miles). At predetermined intervals, the tube was centrifuged at 12 krpm for 5 min using an Eppendorf 5424 microcentrifuge. The supernatant was collected and replaced with an equal volume of fresh 0.9% (w/v) aqueous NaCl solution. After a release period of thirty days, the suspension of remaining particles was completely dissolved in acetonitrile overnight. The concentration of dexamethasone in all collected samples was quantified using UV absorbance at 234 nm against a standard curve of known drug concentrations. The percentage of drug release at each time point was calculated by normalizing the cummulative amount of drug collected at each point with the total amount of drug initially encapsulated in the particles. The release kinetics reported for each particle formulation was obtained from the average of quadruplicate experiments.

2.3. Animal care

The animal protocol was approved by the local animal ethics committees at Massachusetts Institute of Technology (Committee on Animal Care) and Children's Hospital Boston (Institutional Animal Care and Use Committee) prior to initiation of the study. Male SKH-1E mice at the age of 8–12 weeks were obtained from Charles River Laboratories (Wilmington, MA, USA). The mice were housed under standard conditions with a 12-h light/dark cycle at the animal facilities of Massachusetts Institute of Technology, accredited by the American Association of Laboratory Animal Care. Both water and food were provided ad libitum.

2.4. Subcutaneous injection of polymeric microparticles

Before subcutaneous injection of microparticles, mice were kept under inhaled anesthesia using 1–4% isoflurane in oxygen at a flow rate of 2.5 L/min. Lyophilized microparticles with or without encapsulated drug were suspended in sterile 0.9% (w/v) phosphate buffered saline at a concentration of 5 mg/mL. A volume of 100 μL of this suspension was injected subcutaneously via a 23G needle at each of the six spots on the back of the mouse.

2.5. In vivo fluorescent imaging of whole animal

Mice were started on a non-fluorescent alfalfa-free diet (Harlan Teklad, Madison, WI, USA) three days prior to subcutaneous injections of microparticles and maintained on this diet till the desired sacrifice time point for tissue harvesting. The imaging probe ProSense-680 (VisEn Medical, Woburn, MA, USA), at a concentration of 2 nmol in 150 μl of sterile phosphate buffered saline, was injected into the mice tail vein. After 24 h, *in vivo* fluorescence imaging was performed with an IVIS-Spectrum measurement system (Xenogen, Hopkinton, MA, USA). The animals were maintained under inhaled anesthesia using 1–4% isoflurane in oxygen at a flow rate of 2.5 L/min. For monitoring cathepsin activity, whole-animal near-infrared fluorescent images were captured at an excitation of 605 nm and emission of 720 nm and under optimized imaging configurations. A binning of 8 × 8 and a field of view of 13.1 cm were used for imaging. Exposure time and f/stop (the opening size of the aperture) were optimized for each acquired image. Background auto-flourescence of PLGA particles was also imaged at an excitation of 465 nm and emission of 560 nm. Data were analyzed using the manufacturer's Living Image 3.1 software. All images are presented in fluorescence efficiency which is defined as the ratio of the collected fluorescent intensity normalized against an internal reference to account for the variations in the distribution of incident light intensity. Regions of interest (ROIs) were determined around the site of injection. ROI signal intensities were calculated in fluorescent efficiency.

2.6. Tissue harvest and histology processing

At the desired time points, mice were euthanized via CO_2 asphyxiation. The injected microparticles and 1 cm^2 area of full thickness dermal tissue surrounding the implant were excised, placed in histology cassettes and fixed in 10% formalin overnight. Following fixation, the tissues were dehydrated by transferring the cassettes to 70% ethanol solutions. The polymer particles with surrounding fixed tissues were embedded in paraffin and sectioned into samples of 5 μm thickness. These samples were stained with hematoxylin and eosin (H&E) for histological analysis.

2.7. Histology analysis by laser scanning cytometry

The extent of cellular infiltration to injected polymer spots was determined by semi-quantitative imaging cytometry using the iCys Research Imaging Cytometer with iNovator software (CompuCyte, Cambridge, MA, USA). A scanning protocol for quantification was configured with excitation by blue 488 nm laser and a virtual channel for hematoxylin detection. Low resolution tissue scans with the 20x objective were performed to capture preliminary images of all tissue sections in each slide. High resolution tissue scans were subsequently acquired using the 40x objective and step size of 0.5 μm. The threshold in the hematoxylin channel for detection of cell nuclei was optimized to selectively contour individual nuclei. Cross-sectional areas of the polymer spots excluding the dermal and skeletal tissues were defined. The nuclei number and nuclei area measurements were taken from within these regions. The extent of cellular infiltration into each polymer spot was calculated as the ratio of the total nuclei area to total polymer cross-sectional area.

2.8. Statistical analysis

The values of the fluorescent signals and the extent of cellular infiltration were averaged and expressed as the mean ± standard error of the mean. Comparisons of values were performed by the Student's two-tailed two-sample *t*-test. *P* values less than 0.05 were considered significant.