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Spatial and temporal effects of controlled-release anti-inflammatory drug on the cellular dynamics of subcutaneous host reactions

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Abstract: In general, biomaterials induce a non-specific host response when implanted in the body. This response 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 anti-inflammatory drugs on the cellular dynamics of the innate immune response to subcutaneously implanted poly(lactic-co-glycolic) microparticles. Noninvasive fluorescence imaging methods were exploited to investigate the effect of dexamethasone drug loading and release kinetics on the local and systemic inflammatory cellular response. Temporal monitoring of foreign body response by *in vivo* imaging and *ex vivo* histological analysis shows that inhibition of cathepsin activity in the early phase is correlated to decreased cellular infiltration at later phases of the foreign body response. We believe the method developed here may facilitate the design of controlled release platforms for the reduction of the foreign body response to implanted biomaterials.
Introduction

One major challenge to clinical application of biomaterials and medical devices is their potential to induce a non-specific host response\(^1\text{-}^8\). 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\(^1\),\(^8\text{-}^{11}\). Fibrotic cell layers can hinder electrical\(^\text{12}\) or chemical communications and prevent transport of analytes\(^{13}\text{-}^{15}\) and nutrients, thus leading to the eventual failure of many implantable medical devices such as glucose sensors\(^3\),\(^4\),\(^16\), neural probes\(^17\), immune-isolated pancreatic islets\(^{18}\text{-}^{20}\) and biodegradable polymeric stents\(^5\).

Incorporating controlled release delivery systems of anti-inflammatory drugs into medical devices has been proposed to mitigate this host response and improve device durability\(^21\text{-}^{25}\). This approach has shown some promises in clinical applications for humans. For example, controlled elution of steroids from pace-maker electrical leads have been developed to reduce fibrosis formation and enhance long-term electrical communication between the electrical leads and surrounding cardiac tissue\(^12\). However, similar attempts to improve the performance of other medical devices such as implanted glucose sensors\(^26\) and immuno-isolated islets for diabetes therapy have proven challenging\(^1\). There remains a substantial need to better understand the immuno-modulatory effects of these anti-inflammatory drugs on the host-tissue biology at the implant site\(^21\). Such knowledge can lead to better design of appropriate controlled-release drug delivery system to improve the biocompatibility of implanted medical devices.

Development of controlled release formulations to mitigate host responses has largely focused on decreasing the number of inflammatory cells infiltrating the host-device interface. Hickey \textit{et al.} developed a mixed microsphere system containing dexamethasone, a steroidal anti-inflammatory drug, to achieve zero-order \textit{in vitro} release kinetics and suppress tissue response against a thread-induced injury model in rats for up to 1 month\(^27\),\(^28\). Recent studies on a hydrogel composite containing dexamethasone-loaded PLGA particles also suggest that sustained release of the same drug can minimize the inflammatory reactions at the tissue-material interface\(^29\text{-}^{31}\). While these studies have provided valuable information, they only address the effects of these drug delivery systems on the cell types, quantity and distribution via \textit{ex vivo} analysis of excised tissue. However, various factors in the design of controlled release formulations such as the selection of drug, drug loading percentage, particle sizes and corresponding release kinetics can
affect a range of dynamic biological activities in the host response. The presence of anti-inflammatory drugs can alter not only cell types and quantity but also the kinetics of cellular activities such as secretion of inflammatory enzymes or cell signaling pathways. In vivo cellular secretion products might affect the degradation rate of the polymeric matrix used to encapsulate drugs, and are partly responsible for the discrepancy between in vitro and in vivo release kinetics. Therefore, we hypothesize that monitoring the spatial and temporal dynamics of enzymatic activity of the host response in vivo can give 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 cellular dynamics of the host response against subcutaneously implanted polymeric materials. Poly(lactic-co-glycolic) (PLGA 50/50) microparticles with and without dexamethasone were subcutaneously injected dorsally in mice in a six-spot array. Monitoring the in vivo activity of cathepsins, a class of inflammatory proteases, by noninvasive fluorescent imaging reveals that microparticles with low drug loading (1.3%) locally inhibits these enzymes while high drug loading (26%) formulation results in systemic immunosuppression. This low dexamethasone loading is sufficient to attenuate the coverage of the implanted polymer by fibrotic layers. Temporal monitoring of the anti-inflammatory effect by in vivo imaging and ex vivo histological analysis suggests that inhibition of cathepsin activity in the early phase of the foreign body response correlates to decreased cellular infiltration at the later phase of the foreign body response.

2. Materials and Methods

2.1 Fabrication and characterization of PLGA microparticles

Microparticles with or without dexamethasone were prepared using a single-emulsion method with biodegradable PLGA 50/50 (inherent viscosity of 0.95-1.20dl/g) from Lactel (Pelham, AL). Typically, a solution of PLGA and dexamethasone dissolved in dichloromethane, at concentrations of 40mg/ml and 2mg/ml or 30mg/ml respectively, was quickly added to a solution of 50mL 0.5w/v % polyvinyl alcohol and homogenized for 60s at 5000rpm (Silverson L4R, Silverson Machines Ltd., Cheshire, England). The resulting suspension was quickly decanted into 100mL of deionized water and stirred for 30s prior to rotary evaporation (Buchi Rotavap, Buchi, Switzerland) for 3min. The suspension was washed five times by centrifugation at
3000rpm for 3 min. The particles were collected by filtration using 0.2µm 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 spectrofluorometer (Horiba Yvon Jobin, Edison, NJ, USA) with front-face correction. The dexamethasone loading of all microparticles was determined by dissolving 2mg of microspheres in 1mL 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. Briefly, 3mg of dexamethasone-loaded PLGA microparticles of each drug loading were suspended in 1mL of 0.9w/v% NaCl solution in a 1.5mL 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 12krpm 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. The concentration of dexamethasone in the collected supernatant was quantified using UV absorbance at 234nm against a standard curve of drug concentration. After a release period of thirty days, the suspension of remaining particles was completely dissolved in acetonitrile overnight. The release kinetics reported for each drug loading were 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-hour 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, 100ul of lipopolysaccharide at a concentration of 1mg/mL was administered to each mouse via intraperitoneal injection. Lyophilized
microparticles with or without encapsulated drug were suspended in sterile 0.9w/v % sodium chloride at a concentration of 5mg/mL. Mice were kept under inhaled anesthesia using 1–4% isoflurane in 100% oxygen at a flow rate of 2.5L/min. A volume of 100μL 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 until the desired sacrifice time point for tissue harvesting. The imaging probe ProSense-680 (VisEn Medical, Woburn, MA, USA), at a concentration of 2nmol in 150μl sterile PBS, was injected into the mice tail vein. After 24 hours, 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/balance oxygen at a flow rate of 2.5L/min. For monitoring cathepsin activity, whole-animal near-infrared fluorescent images were captured at an excitation of 605nm and emission of 720nm and under optimized imaging configurations. A binning of 8×8 and a field of view of 13.1cm were used for imaging. Exposure time and f/stop – the relative size of the opening of the aperture - were optimized for each acquired image. Background autoflourescence of PLGA particles were also imaged at an excitation of 605nm and emission of 720nm. 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 CO₂ asphyxiation. The injected microparticles and 1cm² 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 spots with surrounding fixed tissues were embedded in paraffin and sectioned into samples of 5μm 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 488nm 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 0.5µm. 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.

3. Results and Discussion

3.1 Spatial effect of controlled-release anti-inflammatory drug

3.1.1 Effect of drug loading on controlled release property

We first investigated the controlled release effect of microparticle formulations with different loadings of anti-inflammatory drug. Dexamethasone, a synthetic steroid, was selected for incorporation into the PLGA microparticles because it is the most potent long-acting glucocorticoid which has been reported to decrease cellular recruitment to biomaterials and minimize fibrotic deposition on FDA-approved pace-maker leads. PLGA particles with or without different drug loadings were fabricated by a water-in-oil emulsion method. Each formulation of drug-loaded particles was tested in triplicate through subcutaneous injections at alternating sites onto the back of hairless, immunocompetent mice as shown in the injection scheme (Figure 1A). Control particles without drug loading were similarly administered at three
symmetrically opposite sites on the same mice. The mice are imaged twenty four hours after intravenous administration of Prosense680, a near-infrared fluorescent probe to detect the activity of cathepsin enzymes which are inflammatory proteases secreted by immune cells\textsuperscript{42-44}. Figure 1B-C shows the imaging results 4 days after the injection for two representative mice corresponding to two particle formulations with low (1.3w/v\%) and high (26w/v\%) drug loadings. For particle formulations with lower drug loading (Figure 1B), cathepsin activity of inflammatory cells were observed at three injection sites with control PLGA particles. These fluorescent signals were absent for the drug-loaded particles at the symmetrically opposite sites. The juxtaposition of cathepsin-absent sites next to discrete neighboring sites with high cathepsin activity suggested that the anti-inflammatory effect was spatially localized at the injection sites of drug-loaded particles. The inhibition of cathepsin at three different positions also verified that the effectiveness of the anti-inflammatory drug is independent of the injection sites. Though the mechanism of action for dexamethasone is not completely understood, it is known to act via a variety of pathways\textsuperscript{33} resulting in attenuation of inflammatory cell cascades when administered systemically\textsuperscript{45}. \textit{Ex vivo} histology studies also reported that this drug decreases fibroblastic recruitment and collagen production at implant sites\textsuperscript{46}. Our data demonstrated \textit{in vivo} for the first time that controlled release formulations of dexamethasone at low drug loadings exhibits specific and localized inhibition of cathepsin activity in host response to subcutaneously implanted materials.

At the higher drug loading (Figure 1C), there appears to be a systematic immunosuppressant effect causing the disappearance of cathepsin signals from all the control particles. This might be due to the significant initial burst release from the particle formulation with higher drug loadings, as shown in the \textit{in vitro} drug release profile (Figure 1D). Some of the mice with high drug loadings died after 7-10 days while mice with lower drug loadings maintained a healthy body condition until the time of sacrifice at 28 days. This understanding of the influence of drug loading on the \textit{in vivo} inhibitory effect is important in selecting appropriate drug delivery formulations for incorporation into medical devices. An appropriate anti-inflammatory drug release is useful to avoid unwanted side effects of systemic circulation while ensuring sufficient mitigation of the host response to improving long-term device performance.

\textbf{3.1.2 Anti-inflammatory drug attenuates coverage of implanted polymer by immune cell layers}
We also imaged the mice with particles of low drug loadings at both the near infrared and visible wavelength conditions (Figure 2) and discovered an interesting phenomenon related to the optical property of the immune cell layers surrounding the injected polymer particles. Figure 2B and 2C displays the two fluorescent signals overlaid on a gray photograph for one representative mouse imaged ten days after subcutaneous injection of particles. PLGA 50/50 particles have strong autofluorescent properties in the green visible wavelengths but insignificant signals in the near infrared region where cathepsin activity is imaged (Supplementary Information SI.2). In figure 2C, the green auto-fluorescence of PLGA 50/50 particles was clearly visualized at all injection spots with drug-encapsulated particles, confirming that the polymer particles are still present at the injection sites. Hence, the absence of cathepsin signals at the drug-loaded polymer spots in figure 2B definitely results from the drug’s inhibitory effect and is not due to the disappearance of degrading polymer. Interestingly, the auto-fluorescence of the control PLGA particles was not as visible, despite the prominent protrusion shown on the gray photographic image. We hypothesize that inflammatory cells are more extensively recruited to these control PLGA 50/50 particles, covering them in more compact cellular layers and reducing their auto-fluorescence at visible wavelengths by tissue scattering and absorption\textsuperscript{47}. This hypothesis was confirmed by \textit{ex vivo} imaging of the excised polymer spots in figures 2E-G. In figure 2E, the near-infrared cathepsin activity of the \textit{ex vivo} tissue is consistent with the \textit{in vivo} data. At the visible wavelength condition in figure 2F, \textit{ex vivo} control particles also show some auto-fluorescence that is not as intense as the drug-loaded particles. This weak auto-fluorescence of the control particles was not seen during \textit{in vivo} imaging as the polymer spots were underneath the skin layer. The colored photograph (figure 2G) of the same \textit{ex vivo} tissue verifies that the control polymer spots are compacted in extensive fibrotic tissue while the drug-loaded microparticles remained flattened against the skin, and covered with minimal fibrotic cell layers. In addition, figure 2D shows the multiplex image combining \textit{in vivo} signals from both near-infrared cathepsin activity and visible auto-fluorescence of the same mouse. There is no overlapping between these two signals suggesting that the fibrotic cell layers completely cover the control polymer particles. Overall, our data suggests that inhibition of cathepsin activity by controlled release anti-inflammatory drug correlates with decreased coverage of the implanted particles with immune cell layers. There exists the possibility for future work in utilizing this
optical property of immune cell layers to noninvasively monitor long term fibrosis against subcutaneously implanted materials.

3.2 Temporal effect of controlled-release anti-inflammatory drug

3.2.1 Time-evolution of cathepsin activity

In vivo host response to implanted materials is a dynamic process that involves many different cell types and biological pathways. Neutrophils, monocytes and macrophages release cathepsins during the process of degranulation\textsuperscript{48, 49}. To kinetically monitor the effect of anti-inflammatory drugs on the activity of these immune cells, cathepsin activity was imaged on days 3, 10, 17 and 28 (Figure 3A) in four replicates administered with particles of 1.3\% drug loading. Figure 3B shows the result for one representative mouse imaged at four different time points after subcutaneous injection of polymer particles. Cathepsin activity in response to the control PLGA 50/50 particles was highest at days 3 and 10 and decreased significantly at later time points. However, for the microparticles containing dexamethasone, such cellular activity was suppressed at earlier time points and remained absent over the entire period of 28 days. Quantification of the time-evolution of this cathepsin activity is presented in figure 3C showing statistically significant differences between the two particle formulations at days 3 and 10. This temporal analysis suggests that monitoring of cathepsin activity is useful in detecting the anti-inflammatory effect of controlled-release therapeutics in the early phase of host response.

3.2.2 Time-evolution of cellular infiltration

To understand how the temporal dynamics of in vivo cathepsin activity is related to time-dependent cellular infiltration within the implanted microparticles, we also performed standard histological analysis of excised tissues. Three or four mice were sacrificed at days 3, 10, 17 and 28 corresponding to the imaging time points in Figure 5. The excised polymer and surrounding tissues were fixed, processed histologically and stained with Hematoxylin and Eosin. Figure 4A shows representative tissue sections in which cell nuclei stained dark blue while collagen and cytoplasmic materials stained pink.

Qualitative evaluation of individual samples reveals that for many samples collected on days 3 and 10, the central portions of the polymer sections were detached during histology processing while the samples for days 17 and 27 remained intact. The non-homogenous properties of the dermal tissue containing polymer particles render it fragile during histological processing steps such as microtome sectioning and exposure to various organic solvents\textsuperscript{50}. In the
earlier phase of the inflammation response, the cellular layers surrounding the implants might be thinner and weaker; hence the samples on days 3 and 10 are more prone to dissociation with the dermal tissue. In the later phase of days 17 and 27, the wound healing response has been resolved\textsuperscript{9} with the formation of fibrotic capsules containing the particles; and thus the samples become more resilient during histology processing.

Despite this lower quality of samples on days 3 and 10, we qualitatively observed neutrophils infiltrating the spaces between the remaining polymer particles for both the control and drug-loaded samples while minimal collagen deposition was present. At the later time points of days 17 and 27, extensive macrophages infiltration and collagen networking were observed throughout the polymer sections of the control samples while the drug-loaded samples were free of cellular infiltration.

We also utilized Laser Scanning Cytometry to quantify the amount of inflammatory cells recruited to the polymer injection sites following established protocols\textsuperscript{25, 37, 51-53}. Figure 4B shows the extent of cellular infiltration into each polymer spot which was calculated as the ratio of the total nuclei area to the total area of the polymer cross-section. The quantitative cellular coverage ratio was not statistically different for days 3 and 10, possibly due to the sample detachment at the earlier time points. However, the extent of infiltration of inflammatory cells is significantly lower for the drug-encapsulated polymers at later time points (days 17 and 27). Together, the histological data and fluorescent imaging provides complementary information to confirm that incorporation of dexamethasone decreases early protease activity and long-term cellular infiltration in the host response against subcutaneously implanted materials.

4. Conclusion

In this study, we have demonstrated the in vivo spatial and temporal effects of controlled-release anti-inflammatory drug on the host response against subcutaneously injected microparticles. Microparticles with low drug loading can locally inhibit the activity of cathepsin enzymes from immune cells while high drug loading formulation results in systemic immunosuppression. We also learned that incorporation of dexamethasone at a low loading of 1.3w/v\% attenuates the coverage of polymeric microparticles by immune cell layers. Temporal monitoring of the drug effect confirms that incorporation of dexamethasone decreases early enzymatic activity and long-term cellular infiltration in the host response against subcutaneously implanted materials.
Better understanding of the effect of anti-inflammatory therapeutics on the host response against implanted materials can potentially aid in the design and development of microsphere systems incorporating bioactive agents. While we only tested one drug here, we can use the same strategy to investigate the effects of different classes of existing drugs or to screen for new small molecules to expand the existing pool of anti-inflammatory inflammatory drugs. Controlled release formulations of such anti-inflammatory therapeutics may be useful for implanted biomedical devices such as cardiovascular stents and glucose sensors.

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References

Figure captions

Fig 1: Effect of drug loading on localization of anti-inflammatory effects. (A) Injection pattern showing administration sites of PLGA particles without (○) and with (■) dexamethasone. (B) Near infrared fluorescent imaging shows a high level of cathepsins at the injection sites of control particles but localized inhibition of these enzymes at the sites of particles with low drug loading. (C) Inhibition of cathepsin activity at all injection sites was observed when particles with high drug loadings were investigated. (D) In vitro release profile of dexamethasone shows a more pronounced initial burst release from microparticles with high drug loadings.

Fig 2: Anti-inflammatory drug attenuates coverage of implanted polymer by immune cell layers (A) Injection pattern showing administration sites of PLGA particles without (○) and with (■) dexamethasone. (B-C) Fluorescent imaging of the same mouse at different wavelengths shows near-infrared signal of cathepsin activity (B) only at the sites of control particles and visible PLGA auto-fluorescence (C) only at the sites of drug-loaded particles (D) Multiplex image combining in vivo fluorescent signals at both wavelengths. (E-F) Fluorescent imaging of ex vivo tissue at different wavelengths shows near-infrared cathepsin activity (E) consistent with in vivo data. At the visible wavelength condition (F), ex vivo control particles also show some auto-fluorescence of lower intensity than drug-loaded particles. (G) Colored photograph of the same ex vivo tissue shows that the control polymer spots are compacted in extensive fibrous tissue which reduces PLGA auto-fluorescence at the visible wavelength. The drug-loaded microparticles remained flattened against the skin with minimal cellular coverage thus retaining their auto-fluorescence.

Fig 3: Quantitative temporal monitoring of cathepsin activity. (A) Timeline of probe administration and imaging. (B) Near-infrared fluorescent visualization of one representative mouse over a period of 28 days demonstrates the inhibitory effect of dexamethasone in the earlier time points. All figures are of the same color scale. (B) Quantification of near-infrared fluorescent signals from four replicates show that cathepsin activity against control microparticles is higher than drug-loaded microparticles at days 3 and 10, (***) indicates P<0.05 by the Student’s two-sample two-tailed t-test.
**Fig 4:** Quantitative monitoring of cellular infiltration to the interparticle spaces. (A) Representative histology sections of excised tissues containing PLGA particles with and without dexamethasone from different mice sacrificed at various time points. Scale bar represents 50um for all pictures. (B) Quantitative analysis of cellular infiltration by LSC shows the inhibitory effect of dexamethasone at later time points: days 17 and day 28. Extent of infiltration by inflammatory cells is defined as the ratio of total nuclei area to the total area of the polymer cross-section. (**) indicates P<0.05 by the Student’s two-sample two-tailed t test.
Figure 1
Figure 2
Figure 3
Figure 4

(A) Histological images of control and Dex/PLGA-treated samples at Day 3, Day 10, Day 17, and Day 28.

(B) Graph showing the extent of infiltration by inflammatory cells over time for PLGA particles and PLGA particles with dexamethasone. ** indicates statistical significance.
Supplementary information

SI 1. Fluorescent properties of PLGA microparticles (A) *In vitro* and *in vivo* fluorescent images of PLGA microparticles with different glycolide monomer contents. When imaged at the visible wavelength configuration (excitation at 465nm, emission at 560nm), microparticles fabricated from PLGA 50/50 show the strongest autofluorescent intensity, both *in vitro* and *in vivo*. No significant signal was detected at the near-infrared imaging configuration used to detect cathepsin activity (excitation at 605nm, emission at 720nm). (B) Excitation and emission spectra of PLGA 50/50 particles in solid state.

![Fluorescent properties of PLGA microparticles](image)

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SI 2: Incorporation of dexamethasone does not alter physical properties of the microparticles. A) SEM images showing similar particle sizes and spherical morphology of control PLGA particles and those encapsulating dexamethasone. All scale bars represent 10 µm. Adjacent fluorescent images show strong auto-fluorescence at visible wavelength and no detectable signal at near-infrared imaging condition for both particle formulations. B) Similar fluorescent excitation and emission spectra of PLGA microparticles with and without dexamethasone.