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Nanoparticles administered intrapericardially enhance payload myocardial distribution and retention

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Abstract

Pharmacological therapies for cardiovascular diseases are limited by short-term pharmacokinetics and extra-cardiac adverse effects. Improving delivery selectivity specifically to the heart, wherein therapeutic drug levels can be maintained over time, is highly desirable. Nanoparticle (NP)-based pericardial drug delivery could provide a strategy to concentrate therapeutics within a unique, cardiac-restricted compartment to allow sustained drug penetration into the myocardium. Our objective was to explore the kinetics of myocardial penetration and retention after pericardial NP drug delivery. Fluorescently-tagged poly(lactic-co-glycolic acid) (PLGA) NPs were loaded with BODIPY, a fluorophore, and percutaneously administered into the pericardium via subxiphoid puncture in rabbits. At distinct timepoints hearts were examined for presence of NPs and BODIPY. PLGA NPs were found non-uniformly distributed on the epicardium following pericardial administration, displaying a half-life of ~2.5 days in the heart. While NPs were mostly confined to epicardial layers, BODIPY was capable of penetrating into the myocardium, resulting in a transmural gradient. The distinct architecture and physiology of the different regions of the heart influenced BODIPY distribution, with fluorophore penetrating more readily into atria than ventricles. BODIPY proved to have a long-term presence within the heart, with a half-life of ~7 days. Our findings demonstrate the potential of utilizing the pericardial space as a sustained drug-eluting reservoir through the application of nanoparticle-based drug delivery, opening several exciting avenues for selective and prolonged cardiac therapeutics.

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Abstract

Keywords

Pericardial drug delivery; nanoparticles; local drug delivery; cardiovascular disease; poly(lactic-co-glycolic acid)

1. Introduction

Limited cardiac specificity of systemically-administered drugs severely dampens therapeutic efficacy and increases adverse side effects. Advances in catheter-based approaches have enabled strategies aimed at local delivery of therapeutics, including intracoronary [1, 2] and intramyocardial [3] administration. Local delivery overcomes shortcomings associated with systemic delivery by amassing drugs at the site of action, leading to high local concentrations, less required dose, and reduced toxicity. Intracoronary approaches are capable of localizing therapeutics specifically within coronary artery walls, but suffer from inconsistent delivery, rapid washout, and insufficient distribution to the myocardium [4, 5]. Intramyocardial delivery results in high myocardial drug deposition [6, 7], but is limited by injury to the muscle and poor local retention of injectate over time [8, 9].

The fluid-filled pericardial sac represents a unique compartment for local drug delivery, a reservoir that allows for transmural migration of therapeutics into the heart. Pericardial drug delivery has several advantages, chief among them being ease of access, proving a more safe, convenient, and reproducible procedure when compared to other cardiac delivery approaches [10]. Higher local drug concentrations can be obtained in the heart, owing principally to lower clearance rates [7, 11]. Consequently, lower doses are required for therapeutic effect, resulting in less systemic exposure of drug, which in turn reduces off-target toxicities [12]. Pericardial delivery has been used to administer a variety of drugs for the treatment of disease, including paclitaxel for inhibition of vascular smooth muscle cell proliferation in restenosis [13], procainamide and digitalis for antiarrhythmic therapy [14], and insulin-like growth factor I for treatment of chronic heart failure [15].

Despite the numerous advantages afforded by pericardial drug delivery, clearance by epicardial vasculature and lymphatics presents a challenge to long-term drug retention, with small molecular weight drugs cleared faster than larger peptides and constructs [16]. Nano- and micron-scale particles, ranging in size from 10–1,000 nm, have been used as local drug

depots to enhance and maintain therapeutic levels of drug within their immediate surroundings [17–19]. Formulating drugs within biodegradable nanoparticle (NP) constructs allows for drug stabilization and solubilization, and importantly, prolonged and controllable release profiles of therapeutics over time. With these advantages in mind, nanoparticle-based drug delivery to the heart has been explored previously. Nanoconstructs have been administered intravenously to target failing [20] and ischemic [21–24] hearts, as well as locally through intramyocardial administration for the treatment of infarcts [25, 26] and intracoronary delivery for the treatment of in-stent restenosis [27].

Our objective was to examine the potential of exploiting the pericardial space as a reservoir for nanoparticle-based drug delivery to the heart (Fig. 1a and b). Combining sustained release of therapeutics from NPs with low clearance from the pericardium was hypothesized to result in a prolonged, site-specific drug-eluting depot for delivery to the myocardium. Fluorescently-tagged poly(lactic-co-glycolic acid) (PLGA) NPs encapsulating a fluorophore, BODIPY, were administered intrapericardially to rabbits and retention and distribution in the myocardium evaluated at different timepoints. PLGA NPs had a sustained epicardial presence for prolonged times, a result of low clearance of the carrier construct from the pericardial space. While NPs did not migrate great distances past the epicardium, BODIPY was capable of permeating deep into the myocardium with pronounced accumulation in the atria and the apex. Both PLGA NPs and BODIPY displayed long-term retention in cardiac tissues, with half-lives on the order of days. Findings from this study highlight the prospect of utilizing pericardial-based delivery of nanotherapeutics for the sustained treatment of a variety of diseases.

2. Materials and Methods

2.1. Chemicals and reagents

Ester-terminated (50:50, 0.26–0.54 inherent viscosity, Mn 15,000 Da) and carboxy-terminated (50:50, 0.52–0.75 inherent viscosity, Mn 17,100 Da) PLGA were obtained from Durect Corporation (Birmingham, AL). BODIPY® 630/650-X NHS Ester (BODIPY) was purchased from Life Technologies (Waltham, MA). Cyanine7 (Cy7) amino was purchased from Lumiprobe (Hallandale Beach, FL). Polyvinyl alcohol (PVA, Mw 9,000–10,000, 80% hydrolyzed) was purchased from Sigma-Aldrich (St. Louis, MO). All organic solvents were purchased from Thermo Fisher Scientific (Waltham, MA).

2.2 Cy7 labeling of PLGA

Briefly, carboxy-terminated PLGA was dissolved in anhydrous dichloromethane (DCM). Pre-dried triethylamine (TEA) in a DCM solution containing EDC•HCL (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride) and NHS (N-Hydroxysuccinimide) was added to the PLGA solution, and after 5 min, Cyanine7 amino dissolved in DCM was added. After 2 h of stirring in the dark, the mixture was concentrated and precipitated with cold ether. The filtered product was then dried under vacuum.

2.3 NP fabrication

PLGA NPs were fabricated using a modified w/o/w emulsion method [28]. Briefly, 100 mg of PLGA (20% labeled with Cy7) were dissolved in 1 mL of DCM, to which 40 µg of BODIPY was added. This solution was then added to a 5% PVA aqueous solution while under constant sonication (QSONICA, Newtown, CT) on ice for the duration of 1.5 min. The resulting emulsion was then added to 20 mL of deionized water and left stirring overnight to allow for evaporation of the remaining organic solvent. The solution was filtered using a 1.2 µm Acrodisc® syringe filter (Pall Corporation, Port Washington, NY), NPs collected by centrifugation (12,000 RPM during 10 min), and washed with deionized water.

2.4 NP characterization

Scanning electron microscopy (SEM) was performed on NPs using a Nova NanoSEM (FEI, Hillsboro, OR). Prior to SEM, NPs were coated with a 5 nm 80/20 Platinum/Palladium coating using a Cressinton sputter coater (Watford, UK). Imaging was performed at a landing energy of 5 keV. Size and zeta potential of NPs were determined by dynamic light scattering (DLS, Malvern Zetasizer, Worcestershire, UK). BODIPY concentration was determined by HPLC (Agilent Technologies, Santa Clara, CA). The mobile phase consisted of a 60:40 ratio of 0.1% trifluoroacetic acid and acetonitrile at a flow rate of 1 mL/min through a ZORBAX 300 Extend-C18 column (4.6 × 100 mm 3.5 µm, Agilent Technologies). The BODIPY peak was detected at an absorption wavelength of 630 nm and a retention of 9 minutes. BODIPY release from NPs in PBS (pH 7.4) at 37°C was examined using a previously described method [29].

2.5 Pericardial administration of NPs

All animal studies were approved by the Institutional Animal Care and Use Committee of the Houston Methodist Research Institute. Female New Zealand white rabbits (2–5 Kg) were used for *in vivo* studies. Rabbits were housed in Tecniplast R-suite caging, with 4,670 cm² (5.03 ft²) of floor space, meeting the requirements set forth by the Guide for the Care and Use of Laboratory Animals: Eighth Edition. Experimental groups (n=4) were assigned sequentially (non-randomized). Non-injected rabbit hearts were used as controls. Briefly, rabbits were premedicated by an intramuscular injection of xylazine (0.75–1.5 mg/kg) and atropine, anesthetized by intravenous injection of propofol (5 mg/kg) and continued on isoflurane inhalation. The subxiphoid area was shaved, sterilized and draped. Under sterile conditions, a 21 Gauge, 7 cm long micropuncture needle (Cook Medical, Bloomington, IN) was advanced under the sternum to reach the pericardial sac in its anterior portion. Omnipaque radiographic contrast (GE Healthcare, Boston, MA) was used to guide entrance into the pericardium, verified by dye spreading into the pericardial sac. A 0.018" guide wire was then inserted in the pericardium and a sheath was advanced over the wire (Fig. 1c and d). After obtaining pericardial access, a 0.5 mL solution containing 180 mg of NPs was injected into the pericardial sac, the sheath was flushed with 0.1 mL of normal saline, and withdrawn. Rabbits were allowed to recover from anesthesia and observed.

2.6 Epifluorescence evaluation of hearts

At 1, 4, and 7 d timepoints after NP administration, rabbits (n=4 per timepoint) were sacrificed and the heart and pericardial fluid were collected. Whole-heart epifluorescence was measured using an IVIS Spectrum In-Vivo Imaging System (PerkinElmer Inc, Waltham, MA). BODIPY was measured at λ_{ex} 605 nm and λ_{em} 660 nm, while Cy7 was assessed at λ_{ex} 745 nm and λ_{em} 800 nm with an exposure time of 0.5 and 1 second respectively.

2.7 Confocal imaging of heart cross-sections

Following *ex vivo* imaging, hearts were sectioned into 4 transversal cross-sections that were embedded in OCT for cryo-sectioning. Transverse sections with a thickness of 10 μm were obtained at three levels of the heart: the atria, mid transversal ventricular sections, and the apex. Slides were fixed with acetone during 20 min and rinsed with PBS, after which a mounting media containing DAPI (Vector laboratories, Burlingame, CA) was added and slides sealed. NPs and BODIPY presence in the heart was assessed by confocal imaging, which was performed using a FluoViewTM 1000 confocal microscope (Olympus Imaging America Inc, Center Valley, PA) at a magnification of 20X. Composite microphotograph stitches, comprising the atrial or ventricular wall were composed of consecutively obtained images spanning the entirety of the myocardium, from the epicardial wall to the endocardium. Stitch length was proportional to the length of the tissue section, ranging from 0.4 mm in the atria to 4.5 mm in the ventricles.

2.8 Image analysis

Confocal images were analyzed using ImageJ software (NIH, Bethesda, MD). Briefly, contrast was subtracted and RGB channels were split. To eliminate areas that did not contain tissue, regions of interest (ROIs) were generated by treating nuclear staining as an object and enlarging the selection by 9 μm . In order to evaluate BODIPY and NP presence in the myocardium, ROIs from ventricles and atria were measured in 500 and 100 μm windows respectively, from the epicardium to the endocardium of each particular section. For purposes of detail enhancement, the first 1 mm of each section was evaluated at 250 μm intervals in ventricles, while the first 0.2 mm of sections were evaluated at 50 μm intervals in atria. Area fraction (Figs. S1 and S2) was quantified with respect to DAPI, and the integrated intensity (Figs. S3 and S4) was obtained as follows:

$$\text{Integrated Intensity} = \frac{\text{"X" Area Fraction}}{\text{DAPI Area Fraction}} * \text{"X" Intensity}$$

where "X" represents the dye of interest (Cy7 or BODIPY).

2.9 BODIPY quantification in pericardial fluid

BODIPY was extracted from pericardial fluid samples through dilution in 6X volume of methanol followed by vortex and centrifugation. Supernatant was collected and concentrated by vacuum. Dry samples were dissolved in acetonitrile for HPLC analysis. Calibration standards were prepared using BODIPY solutions and pericardial fluid samples from non-treated rabbits. HPLC was performed on samples using the previously described method.

2.10 Pharmacokinetic estimation based on fluorescence

Fluorescence averages in heart tissue at different timepoints were plotted against time for individual heart regions (e.g. left ventricle, right atrium, etc.) and for the whole heart. A single exponential model was fit for PLGA fluorescence approximating clearance from a bolus injection. A double-exponential model was used to fit BODIPY fluorescence assuming a two-compartment model with BODIPY absorption and clearance phases in myocardium. Fitted fluorescence profiles were used to estimate the area under the curve (AUC) and time constants of fluorescence changes, which were treated as surrogate markers for BODIPY and PLGA concentrations in heart.

2.11 Statistical Analysis

Differences between groups were analyzed using One Way ANOVA followed by Post hoc Bonferroni analysis using GraphPad Prism software (GraphPad, San Diego, CA).

3. Results

3.1 1 NP characterization

BODIPY-containing PLGA NPs were spherical and monodisperse in size, as visualized by SEM (Fig. S5a). NPs measured 190 ± 49 nm in diameter as determined by DLS, with a polydispersity index (PDI) of 0.046 and a zeta potential of -37.5 ± 7.1 mV (Fig. S5b). *In vitro* examination of BODIPY release from PLGA NPs showed an initial release of ~12% within the first 24 h, followed by a slow and sustained release of BODIPY over the course of almost 12 d (Fig. S5c). Prolonged release of BODIPY, due to the high viscosity of PLGA and the hydrophobicity of the agent [30], proved ideal for our purposes of examining long-term drug delivery to the heart.

3.2 PLGA NP and BODIPY distribution on the surface of the heart

Delivery of NPs was well tolerated, with rabbits exhibiting no symptoms or complications during or after the procedure. Upon sacrifice and exposure of the thoracic cavity, hearts exhibited no signs of edema, bleeding, or inflammation. Epifluorescence examination of hearts demonstrated elevated fluorescence intensity associated with NPs (Cy7) throughout distinct regions of the heart 1 d following pericardial delivery (Fig. 2a). At timepoints of 4 and 7 d, fluorescence remained prominent, albeit, at decreased levels compared to day 1. At 1 day, heightened fluorescence signal emanated from atrial and interventricular regions, while at later timepoints, higher fluorescence emanated from the atria and apex. Quantification based on radiant efficiency demonstrated an approximate 34% decrease in fluorescence associated with PLGA NPs at later timepoints compared to day 1 (Fig. 2b). Epifluorescence findings of NP distribution were compared to average fluorescence intensities obtained from confocal microscopy examination of the epicardial surface (50 μ m) of different regions of the heart. High NP fluorescence was observed at 1 d in the epicardium, with signal decreasing over time (Fig. 2c), a pattern that correlated with epifluorescence results (Fig. 2d). However, the decrease observed after day 1 was much more pronounced in the epicardium than on the surface of the heart, indicating that NPs were mostly confined to the pericardial space and surface of the heart.

In contrast to PLGA NPs, BODIPY exhibited a more rapid clearance from the heart surface, decreasing significantly by 4 and 7 d timepoints (Fig. 3a and b). At day 1, fluorescence of BODIPY was high, with distribution correlating with regions of NP fluorescence, a pattern preserved at days 4 and 7 despite a stark decrease in intensity at these timepoints. Average radiant efficiency revealed an approximate 70% decrease in epifluorescence from day 1 to day 4, with minimal change observed between days 4 and 7 (Fig. 3b). Once more, epifluorescence was compared to confocal microscopy examination of epicardial layers of different regions of the heart. Confocal imaging of the epicardium showed a decrease in BODIPY average fluorescence intensity over time (Fig. 3c), a pattern that once again correlated with epifluorescence findings (Fig. 3d). However, fluorescence did not decline as precipitously in tissue as it had on the surface of the heart, rather undergoing a gradual decrease, indicating that at later timepoints, BODIPY was principally found to have penetrated into the heart. The decrease of BODIPY in the pericardial space is supported by LC/MS evaluation of pericardial fluid at different timepoints. While initially high at day 1, BODIPY concentration in the pericardial fluid significantly decreased by days 4 and 7 (Fig. S6).

3.3 NP and BODIPY myocardial distribution

NPs were capable of penetrating into the heart; however, due to their large size, they did not undergo extensive transmyocardial migration, remaining confined to epicardial layers (Figs. 4 and 5; Fig. S7). Higher amounts of NPs were found in atria compared to ventricles. Within the left atrium, high NP fluorescence was detected within the first 150 μm of the heart on day 1 and 4. Fluorescence intensity gradually decreased with increasing distance from the epicardium at day 1, leveling off ~ 500 μm from the surface. At day 4, NP intensity was higher within the first 150 μm compared to day 1, gradually diminishing at a distance of 200 μm from the epicardium. While NP fluorescence within the myocardium at day 7 was reduced compared to prior timepoints, intensity did not decrease at further distances from the heart. The epicardium of the right atrium also contained high levels of NP fluorescence at day 1, with intensity gradually diminishing at a distance of 200 μm from the surface. A similar pattern of distribution was observed at days 4 and 7, albeit, with a pronounced reduction in intensity in the epicardium.

NP fluorescence was high within epicardial layers of the left ventricle in the mid (between apex and base) region of the heart, decreasing in intensity in a time- and distance-dependent manner (Figs. 4 and 5; Fig. S7). After ~ 1 mm, NP presence was minimal within the myocardium. Confocal examination of the apical left ventricle at day 4 showed much reduced intensities compared to the mid left ventricle, with fluorescence diminishing at shorter distances from the surface. Comparatively, the right ventricle had less fluorescence than the left ventricle.

BODIPY released from NPs was detected at high amounts over the entire myocardium within atria, with fluorescence decreasing in a time- and distance-dependent fashion (Figs. 4 and 6; Fig. S7). BODIPY intensity was found at elevated levels and considerable distances from the epicardium at day 1 in the left atrium, with fluorescence observed at a distance of ~ 500 μm into the myocardium. At timepoints of 4 and 7 days, BODIPY concentration in the

epicardium was reduced compared to day 1, and the amount and penetration distance in the myocardium was decreased. In the right atrium, BODIPY at the epicardial surface was high and equivalent across all timepoints. Distribution within the myocardium was similar at days 1 and 4, displaying a gradual decrease as a function of distance from the myocardium. Despite high fluorescence intensity in the epicardium, BODIPY fluorescence diminished at shorter distances from the epicardium compared to days 1 and 4 (Fig. 6).

BODIPY fluorescence within the mid left ventricle was high at day 1 compared to days 4 and 7 (Figs. 4 and 6; Fig. S7). Intensity in the mid left ventricle remained constant throughout the length of the myocardium at all timepoints, despite a substantial reduction in BODIPY concentration in the epicardium at later timepoints. BODIPY fluorescence in the epicardium and myocardium of the apical left ventricle was much higher than that in the mid left ventricle across all timepoints (Fig. 6). Epicardial intensity at days 1 and 4 were equivalent, but displayed varying penetration kinetics. Overall, BODIPY decreased with increasing distance prior to 1 mm, after which concentrations leveled off throughout the remainder of the myocardial wall. Fluorescence was reduced in the epicardium and myocardium at day 7 compared to prior timepoints. Similar to findings regarding NPs, BODIPY fluorescence in the epicardium of the right ventricle at all timepoints were much lower than those observed in the left ventricle (Fig. 6). At day 1, BODIPY intensity decreased with increasing distance from the surface, with amount remaining constant throughout the myocardium after approximately 1 mm. BODIPY intensity at day 4 initially decreased, but subsequently increased with increasing distance from the epicardium. Similar to findings from other regions, intensity at day 7 was reduced in the epicardium and myocardium.

3.4 Pharmacokinetics of PLGA NPs and BODIPY within the heart

NPs exhibited a clearance half-life ($t_{1/2}$) in the overall heart of 2.5 days (Fig. 7a, Table S1), with NP presence projected in the heart up to 2 weeks after pericardial administration. Retention of NPs varied among the different regions of the heart (Fig. S8, Table S1), with longer half-lives observed in the atria and medial left ventricle (Fig. 7b). BODIPY reached its maximum concentration within the overall heart at ~1.4 days (t_{max} , Table S2). At day 7, slow clearance occurred in the myocardium, when BODIPY concentration was at its half-life based on fluorescence profile analysis. BODIPY proved long-lived in the heart, with estimation based on fluorescence fitting projecting detectable BODIPY concentrations for more than two weeks in cardiac tissue (Fig. 7a). As in the case of NPs, the kinetics of BODIPY varied from region to region, with BODIPY remaining at the highest concentration for longest in ventricles and the left atria (Fig. 7c).

4. Discussion

Local drug delivery strategies to the heart aim to address the low bioavailability and adverse side effects stemming from systemic administration. As an example, Laham and coworkers demonstrated that less than 1% of basic fibroblast growth factor (bFGF) was found in the myocardium 1 h after intravenous administration, decreasing precipitously to less than 0.1% after 24 h, with a large amount accumulated in the liver [31]. While refinement of catheter-

based interventions have enabled intracoronary [32, 33] and intramyocardial [34] delivery, significant limitations persist. These include inconsistent administration and poor retention due to injectate leakage and rapid clearance into systemic circulation [9, 35]. Pericardial delivery provides a safe, minimally-invasive, and reproducible strategy to localize therapeutic agents within a compartment that in healthy humans can accommodate a volume of ~50 mL [36], allowing for prolonged exposure of drug capable of migrating into cardiac tissue [37]. Herein, we aimed to exploit the pericardial space as a reservoir for local nanoparticle-based drug delivery specifically to the heart (Fig. 1a and b).

The unique location and physiology of the pericardial sac makes it ideal for site-specific drug delivery to the heart, capable of maintaining a sustained presence of therapeutics at high local concentrations. The isolated, fluid-filled pericardial cavity is encased between an inner serous membrane facing the heart, and an outer fibrous layer [38]. The external layer, consisting of the parietal layer of the serous pericardium and the fibrous pericardium, and the internal visceral layer, consisting of mesothelial cells, act as barriers that restrict transport of administered substances out of the pericardial sac. Herein, polymer NPs were found distributed, non-uniformly, on the surface of the heart at different timepoints (Fig. 2). Heightened NP presence was observed in atria and interventricular regions at early timepoints, and the atria and the apex of the heart at later timepoints. This agrees with findings from Santamore et al., who showed that at early timepoints, lissamine green dye localized in interventricular and atrioventricular grooves [39], and March and coworkers, who showed that ^{131}I -labeled diazeniumdiolated albumin (NONO-albumin) accumulated laterally and inferoapically in pigs following pericardial delivery [40]. NP distribution is likely multifactorial, affected by distribution of pericardial fat, which is abundant in the interventricular regions and atrioventricular groove, close to atrial appendages, by anatomical association or entrapment with large vessels and atrial appendages [40], and by apical displacement by the beating motion of the heart [41].

A prolonged exposure of drug is required for improved patient outcomes in a variety of cardiovascular diseases, oftentimes necessitating daily dosage of highly potent drugs with severe side effects. As an example, patients with cardiac arrhythmias are administered a daily dose of amiodarone equivalent to 200 mg [42]. Unfortunately, low bioavailability in the heart, as well as lung, liver, thyroid, eye and skin toxicities [43], offset the clinical benefits of the drug. Herein, sustained maintenance of NPs on the epicardial surface for prolonged timepoints was principally owed to confinement within a compartment with an established low rate of clearance [14]. The turnover rate of pericardial fluid can average 6 h in sheep [44], with epicardial vasculature and lymphatics driving clearance [45]. And while mechanistically unclear, size, charge, tissue affinity, and lipophilicity can play a role in clearance from the pericardial sac [16], with larger molecular weight agents undergoing slower clearance through lymphatic drainage. The NPs used in this study measured ~190 nm (Fig. S5), and were significantly larger than small molecular weight proteins and drugs that undergo clearance by vasculature and lymphatics. The relatively large size of the delivery platform resulted in delayed lymphatic drainage, contributing to long-term retention within the pericardial sac. It is important to note that NPs did not diffuse extensively into the myocardium, remaining largely confined to epicardial layers (Figs. 4 and 5; Fig. S7), which was also a result of the relatively large size of the NPs and the fact that healthy heart muscle

is comprised of dense and tightly-connected tissue that restricts transport of substances into the myocardium. Regardless of limited penetration into the myocardium, NPs had a prolonged presence, with a half-life of approximately 2.5 d in the entire heart (Fig. 7). Design considerations such as incorporation of bioadhesive polymers onto the surface of nanoparticles to promote binding with biological tissues [46, 47], functionalization with ligands that in other applications have been shown to anchor nanoparticles in place [48], and the use of excipients that result in *in situ* cross-linked gels or scaffolds that immobilize nanoparticles [8, 49, 50], can potentially result in long-term localization in the heart following pericardial administration. Combined with nanoparticles engineered to exhibit slow drug release kinetics, the pericardium can function as a sustained release drug-eluting depot for prolonged drug delivery to the heart. Recently, Hualong and coworkers demonstrated neointimal hyperplasia inhibition through sustained rapamycin delivery from a pericardial patch [51], successfully limiting smooth muscle cell migration and hyperplasia.

A variety of drugs have been administered intrapericardially in hopes of countering poor myocardial accumulation observed with systemic and local drug delivery strategies to the heart. Grossman et al. showed significant leakage, as well as rapid clearance of injected material, following direct myocardial injection [9]. Similarly, Lazarous and coworkers demonstrated that intravenous and intracoronary delivery of bFGF resulted in an approximate 0.5% and 4% uptake, respectively [52]. Short residence times of nano- and microscale particles in the heart have also been observed following intravenous [22, 23], intramyocardial [53], and intra-arterial [54] administration. In contrast, a 19% accumulation of bFGF was found in the heart following pericardial administration. Consequently, pericardial delivery has been used to deliver several drugs for treatment of diseased coronary arteries, myocardium, and autonomic nerves [10]. Hou et al. demonstrated that a single-dose pericardial administration of paclitaxel inhibited smooth muscle cell proliferation in a porcine coronary balloon injury model, significantly reducing restenosis [13], while Sideris and coworkers found that pericardial delivery of digoxin reduced the QTc interval, suggesting electrophysiological effects [14].

Despite favorable pharmacokinetics and actions of drugs on distinct targets, long-term retention of intrapericardially-administered agents is compromised by lymphatic and epicardial vasculature clearance. In the aforementioned study by March and coworkers, NONO-albumin delivered intrapericardially had a $t_{1/2}$ of ~22 h [40]. Nanoparticle-based delivery was expected to enhance the local pharmacokinetics and bioavailability of drugs in the heart through prolonged retention in the pericardial sac and sustained release of therapeutics over time. Herein, BODIPY, a fluorophore serving as a model drug released from NPs, was present within different regions of the heart over prolonged timepoints and at considerable myocardial depths from the pericardium (Fig. 6). BODIPY displayed a transmyocardial gradient in all regions of the heart. While NPs were mainly confined to epicardial layers due to size, BODIPY diffused into ventricular and atrial myocardial regions, displaying a sustained presence in these tissues. Indeed, the half-life of BODIPY throughout the entirety of the heart was estimated to be 7 d (Fig. 7).

Penetration of different drugs within distinct regions of the heart after pericardial delivery can significantly impact therapeutic applications. As an example, supraventricular

arrhythmias arise in the atria, while ventricular arrhythmias involve lower regions of the heart. Herein, higher amounts of BODIPY were observed in the atria than in ventricular tissue (Fig. 6), agreeing with findings by Mehra and coworkers, wherein atrial distribution of the antiarrhythmic procainamide was reported to have profound electrophysiological effects, slowing atrial conduction velocity [16]. BODIPY was able to permeate more into atrial tissue principally due to its thin architecture and reduced blood flow compared to ventricular tissue, which possess an abundant supply of vessels in the epicardium that can clear small molecular weight agents [16]. While BODIPY concentration in ventricles was comparatively less than that in atria, ventricular tissue penetration of BODIPY was not severely compromised, with sufficient drug migrating into the myocardium, especially at later timepoints. Drug diffusivity into the myocardium, akin to clearance from the pericardial sac, is dependent on drug physicochemical properties such as hydrophobicity and charge. Interestingly, the hydrophobic nature of BODIPY, as well as the potential for binding, did not affect the penetration of the fluorophore into ventricular myocardium, mirroring findings by Zipes and coworkers, wherein amiodarone, a highly lipophilic drug, was found to migrate transmurally and have profound effects on ventricular electrophysiology [55]. Taken together, the potential of nanoparticle-based drug delivery from the pericardium to act upon different regions of the heart opens several exciting avenues for the treatment of a large variety of disease conditions with highly specific sites of action.

5. Conclusion

We highlight the potential of nanoparticle administration in the pericardial space as a viable strategy for local and sustained delivery of therapeutics to the heart. Our findings demonstrate long-term presence of nanoparticles in the heart following pericardial delivery, with extensive intramural penetration and maintenance of released agent. Nanoparticle-based release in the pericardium can result in a local drug-eluting depot proximal to the heart, which in turn can enhance drug pharmacokinetics, leading to higher site-specific concentrations for longer periods of time, the need for lower concentrations of drugs, and a decrease in systemic side effects. Future work involves the administration of nanoparticles containing clinically-approved drugs, as well as genetic material, to determine the effect of pericardial delivery on conditions such as arrhythmias and heart failure. We will also evaluate this approach in disease models to determine the effect of pathophysiological features of disease on drug and nanoparticle penetration and retention. With its convenience, reproducibility, and safety, pericardial nanoparticle-based drug delivery can greatly impact the treatment of a variety of cardiovascular diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Pericardial administration of NPs for local, site-specific drug delivery to the heart
a) The pericardial space can be readily accessed with a needle, after which cannulation allows for direct delivery of NPs (yellow) within the compartment. **b)** NPs (yellow) distribute within the pericardial sac, whose low clearance rate contributes to a sustained release drug reservoir for prolonged exposure of therapeutics to the heart. **c)** X-ray fluoroscopy image highlighting access of the pericardial space in a rabbit with a micropuncture needle advanced under the sternum, administration of iohexol to direct pericardial entrance, and insertion of a guide wire. **d)** Radiographic contrast present in the pericardial sac following the procedure.

Figure 2. Fluorescence imaging of PLGA NPs on the surface of the heart following pericardial administration

a) Representative epifluorescence images of PLGA NPs overlaid on photographs of whole hearts at different timepoints after pericardial delivery. **b)** Average radiant efficiency of NP epifluorescence at different timepoints. **c)** Average fluorescence intensity of NPs within the first 50 μm of all four cardiac chambers obtained by confocal imaging of transversal cross sections of the heart. **d)** Correlation between NP epifluorescence and average fluorescence intensity from confocal imaging. Results shown as mean $\pm$ SEM.

Figure 3. Fluorescence imaging of BODIPY on the surface of the heart following pericardial administration

a) Representative epifluorescence images of BODIPY overlaid on photographs of whole hearts at different timepoints after pericardial delivery. **b)** Average radiant efficiency of BODIPY epifluorescence at different timepoints. **c)** Average fluorescence intensity of BODIPY within the first 50 μm of all four cardiac chambers obtained by confocal imaging of transversal cross sections of the heart. **d)** Correlation between BODIPY epifluorescence and average fluorescence intensity from confocal imaging. Results shown as mean $\pm$ SEM.

Figure 4. Epicardial and myocardial distribution of PLGA NPs and BODIPY

Representative confocal micrographs demonstrating fluorescence of PLGA NPs and BODIPY in the first 250 μm from the epicardial surface of left atrium, ventricle, and apex at different timepoints following pericardial administration. Blue, red, and green represent DAPI, PLGA NPs, and BODIPY, respectively. The scale bar represents 50 μm .

Figure 5. Fluorescence intensities of PLGA NPs within distinct cardiac chambers following pericardial delivery

Average NP fluorescence intensity distribution, as obtained via confocal microscopy examination of stitches in atrial and ventricular myocardial tissue at distinct timepoints following pericardial administration. Results shown as mean $\pm$ SEM.

Figure 6. Fluorescence intensities of BODIPY within distinct cardiac chambers following pericardial delivery

Average BODIPY fluorescence intensity distribution, as obtained via confocal microscopy examination of stitches in atrial and ventricular myocardial tissue at distinct timepoints following pericardial administration. Results shown as mean $\pm$ SEM.

Figure 7. Local pharmacokinetics of PLGA NPs and BODIPY in the heart following pericardial delivery

a) Normalized PLGA NP and BODIPY fluorescence in the overall heart as a function of time, with their respective half-lives ($t_{1/2}$) displayed. **b)** Half-life ($t_{1/2}$) of PLGA NPs in distinct regions of the heart, represented as a sphere. **c)** The amount of time for BODIPY to reach the maximum concentration (t_{max}) in distinct regions of the heart, represented as a sphere. Darker regions represent areas of heightened $t_{1/2}$ and t_{max} values. RA and LA represent right and left atriums, RV and LV represent left and right ventricles, and RAp and LAp represent right and left apex.