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Tissue response and retention of micro- and nanosized liposomes in infarcted mice myocardium after ultrasound-guided transthoracic injection

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ABSTRACT

Different carrier systems have been investigated for myocardial delivery of biopharmaceuticals for heart disease. Here, we aimed to evaluate the heart retention and tissue response of liposomes intended for cardiac drug delivery. Liposomes were produced by the lipid thin film hydration method followed by sonication. Cytocompatibility tests were performed in murine L929 fibroblasts and H2c9 cardiomyocytes using the Alamar Blue assay. *In vivo* experiments were assessed in a model of myocardial infarction induced by isoproterenol in mice. Cardiac delivery of fluorescent liposomes (rhodamine B-labeled) with different mean sizes (165 nm, 468 nm, 1551 nm and 1954 nm) was performed by ultrasound-guided transthoracic injection. After three days, mice were euthanized for histological evaluation using optical and fluorescence microscopy. No cytotoxic lipid concentrations were determined in the range 9.3 – 120 μM for fibroblasts and cardiomyocytes exposed to liposomes. *In vivo*, large liposomes induced significant inflammation in myocardium compared with the control group ($p < 0.0001$). In contrast, heart mice injected with 468 nm-sized liposomes exhibited a lower number of inflammatory cells. Still, a greater tissue retention 72 h post-injection was found. Therefore, this study demonstrated the feasibility of the echocardiography-guided percutaneous injection to deliver liposomes successfully into the myocardium in a minimally invasive manner. In addition, these findings indicate the potential of liposomes as carriers of biopharmaceuticals for myocardial delivery, supporting the development of further research on these delivery systems for heart disease.

1. Introduction

Cardiovascular diseases (CVD) are the leading cause of global mortality and a major contributor to disability. According to the World Health Organization (WHO), an estimated 17.9 million people died from CVD in 2019, representing 32% of all global deaths [1]. Among the CVD, ischemic heart disease (IHD) has become the leading contributor to the global burden of diseases, responsible for high disability adjusted life years [2]. In the spectrum of IHD, myocardial infarction (MI) is the most important manifestation associated with high morbidity and mortality,

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requiring pharmacological intervention, surgical reperfusion and revascularization procedures (e.g. angioplasty, catheterization). In more complex cases, heart transplantation is the last resort for IHD patients when all other approaches have failed [3,4]. Although advanced revascularization strategies have been contributed to a marked reduction in mortality for CVD, a significant number of patients are not suitable for these procedures or achieve incomplete revascularization. In addition, these treatments fail to repair the injured myocardium after MI. To compound the problem, heart failure is alarmingly emerging as a prominent longer-term illness with a very high mortality rate (>40%) among patients with cardiogenic shock after acute MI, and it remains a particular challenge [5,6].

In this scenario, the development of novel therapeutic strategies for treatment and prevention of MI is crucially required. In last years, a growing investigation has been dedicated to gene therapy [7], cell therapy [8] and the use of growth factors [9]. These innovative approaches have been challenged by hindrance to achieve the heart tissue and promote therapeutic benefits. For example, angiogenic growth factors clinically failed due to the short circulating half-life and high instability of proteins when systemically administered [10]. In turn, stem cell-based therapies still depend on delivery platforms and cytokine stimuli to maintain cell retention and viability after transplantation [11,12]. Therefore, the clinical translation of emerging therapies using biopharmaceuticals such as plasmids or recombinant proteins or even stem cells depend on new technologies for myocardial-targeted delivery.

Different carrier systems have been investigated as potential platforms for delivering biopharmaceuticals to the myocardium for the treatment of MI [13,14]. Among them, hydrogels may favor positive tissue remodeling in association with therapeutic proteins or growth factors [15]. In turn, polymeric micro- and nanoparticles have shown great potential to deliver cytokines into the cardiac tissue, administered via intramyocardial injection in a rat MI model [16,17]. Liposomes are another example of potential drug carrier for myocardial delivery [18–20].

Beyond the choice of the delivery platform, the administration route is another critical factor for reaching the myocardium in a safe and effective manner. In fact, drug therapies for CVD are limited by short-term pharmacokinetics and off-target adverse effects. For instance, intracoronary procedures are capable of localizing therapeutics within coronary artery walls, but suffer from inconsistent delivery, rapid washout, and poor distribution to the heart [21,22]. On the other hand, intramyocardial route (IM) allows high availability and tissue retention [21], but invasive procedures such as thoracotomy or sternotomy may limit its clinical value. Alternatively, ultrasound-guided transthoracic intramyocardial injection is a feasible method able to access specific regions of the heart. Importantly, this approach eliminates the inherent complications of thoracotomy and surgical morbidity besides allowing the use of therapeutic regimens with multiple administration [23].

Liposomes have been used as convenient delivery vehicles for biologically active molecules. These carriers can incorporate both hydrophilic and lipophilic compounds, located in the aqueous compartment and inserted or absorbed into the membrane lipidic, respectively [24]. The release kinetics and targeting properties of liposomes can be refined, and this ability seems to be essential to deliver therapeutics precisely to the infarcted heart [25]. However, the potential of liposomes for myocardial drug delivery has not been extensively investigated.

It was therefore the aim of this study to evaluate the heart retention and tissue response of liposomes administered through ultrasound-guided transthoracic injection. For that, a pharmacologically induced mice model of MI was used.

2. Material and methods

2.1. Material

Soybean phosphatidylcholine (Lipoid S100®) was purchased from Lipoid GMBH (Ludwigshafen, Germany) 7-Hydroxy-3H-phenoxazin-3-one-10-oxide sodium salt (Resazurin sodium salt, M_w: 251.17) was supplied from Sigma-Aldrich (Oakville, Canada). Dulbecco's Modified Eagle Medium (DMEM) to cell culture was furnished by Gibco (Grand Island, NY, USA). Cholesterol, Rhodamine B isothiocyanate, pararosaniline chloride and isoproterenol hydrochloride were supplied by Sigma-Aldrich (Sigma-Aldrich, USA). The cellulose hydrogel 0.8% was obtained by hydration of microcrystalline bacterial cellulose using sterile water for injection USP (Polisa®, Recife, Brazil). Disposable hypodermic syringes containing the cellulose hydrogels were supplied as single units wrapped and sealed with surgical paper previously sterilized by gamma rays. All other chemicals were of analytical grade and used as received.

2.2. Preparation of liposomes

Fluorescence-labeled liposomes (Rhod-Lipo) were prepared using lipids at 120 mM (soybean phosphatidylcholine and cholesterol, 8:2) and rhodamine B (0.5 mg/mL) by the thin film method [26]. Briefly, lipid constituents and rhodamine B were dissolved in a mixture of chloroform and methanol ($3{:}1_{v/v}$) under magnetic stirring. The solvents were removed by using a rotary evaporator at 37 °C and 555 mmHg vacuum pressure. Next, the thin film formed was hydrated with 10 mL of trehalose (10%) in phosphate buffer solution (PBS, pH 7.4), resulting in large multilamellar vesicles (MLVs).

Liposome batches with different sizes at micro- and nanoscale were prepared using an ultrasound probe in order to evaluate their retention in myocardium and biocompatibility. Therefore, liposomes were prepared according to sonication parameters which ranged in the power (1–5) and time (10–150 s). Lastly, liposomes were frozen at − 80 °C, lyophilized and stored at 4 °C before use. Prior to *in vivo* studies, lyophilized liposomes were reconstituted in pH 7.4 phosphate buffer solution and incorporated in a cellulose-based hydrogel (1:1, v/v). The injectability of liposomes incorporated in hydrogel was assessed by their ability to pass through a 29-gauge needle used for heart injection [27,15].

2.3. Characterization of liposomes

Lyophilized liposomes were reconstituted in PBS, pH 7.4, and characterized in terms of particle size, polydispersity index (PDI) and surface charge (zeta potential) using photon correlation spectroscopy (PCS) and electrophoretic mobility techniques, respectively (Particle Analyzer™ Delsa Nano S, Beckman-Coulter, USA).

2.4. Cytotoxicity of liposomes

Murine L929 fibroblast and H9c2 cardiomyocytes were used for evaluating the cytotoxicity of liposomes. Both cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (Cultilab, Campinas, Brazil) and 50 μg/mL gentamicin (Novafarma, Anápolis-GO, Brazil). Fibroblasts and cardiomyocytes were plated into 96-well culture plates at densities of 10^4 cells/well and 5×10^3 cells/well, respectively. Cells were maintained in an incubator containing 5% CO_2 at 37 °C for 24 h. After incubation, liposomes with lipid concentrations at 9.3, 15, 25.9, 43.2, 72, 120 μM were added to the cells and incubated for 72 h. DMEM was used as positive control and pararosaniline chloride (*p*-RANIL, 10 μM) as negative control. The cell viability was analyzed using resazurin sodium salt reagent (10%) after incubation for 4 h. Metabolic activity of cells was determined by measurements of reaction products using a multiplate reader at 570 to 600 nm. In a separated set of experiments, the cytotoxicity of the hydrogel was also assessed in H9c2 and L929 cells using the aforementioned conditions. For that, cells were incubated with hydrogel at the concentrations ranging from 5.3 μg/mL to 170 μg/mL.

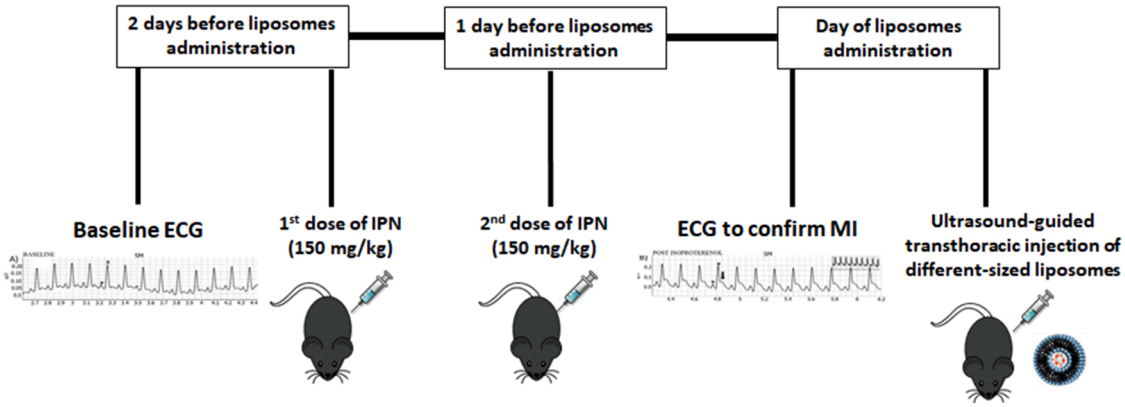

Fig. 1. Experimental design of induction of MI performed through subcutaneous administration of isoproterenol hydrochloride (IPN) at 150 mg/kg/day for two consecutive days before administration of liposomes with different sizes.

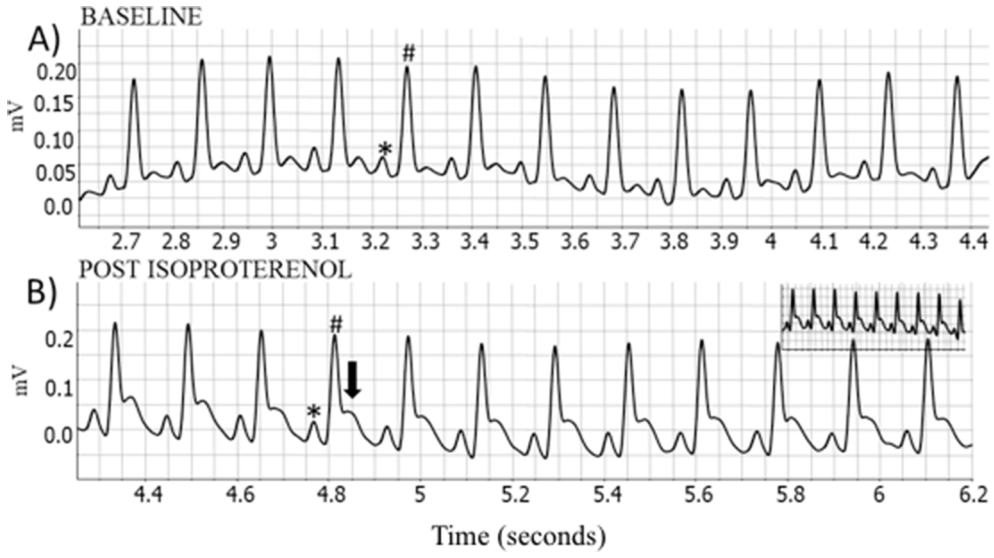

Fig. 2. Representative ECG of a mouse subjected to the isoproterenol-induced myocardial infarction (s.c. 150 mg/kg/day). Baseline ECG (A) and after infarction (B). A significant ST elevation indicates MI (black arrow). #QRS complex. *P wave.

2.5. *In vivo experiments*

2.5.1. *Myocardial infarction model*

The investigation was carried out in agreement with the ARRIVE guidelines (https://arriveguidelines.org/) and EU Directive 2010/63/EU for animal experiments. All procedures involving animals were approved by the Ethics Committee on Animal Use (CEUA) of the Gonçalo Moniz Institute, FIOCRUZ Bahia (Certificate number 010/2015). A total of 17 male C57BL/6 mice (25–30 g) were supplied by the Center for Biotechnology and Cell Therapy Animal Facility, Hospital São Rafael (Salvador, Brazil). Animals were maintained with food and water available *ad libitum* and experiments were carried out using a model of isoproterenol (IPN) induced-myocardial infarction [29,30]. The induction of MI was performed through subcutaneous administration of isoproterenol hydrochloride (IPN, Sigma, St. Louis, USA) at 150 mg/kg/day for two consecutive days according to the experimental design (Fig. 1).

2.5.2. *Electrocardiography*

For electrocardiograms (ECG) recordings, mice were anesthetized with inhaled isoflurane (0.5–2%). ECG acquisition was performed using a bipolar I and II lead, obtained from the Bio Amp PowerLab System (PowerLab 2/20; ADInstruments, Castle Hill, NSW, Australia), allowing for the recording of biological signals in animals with complete electrical isolation. Recordings were bandpass-filtered (1–100 Hz) to minimize environmental signal disturbances at a sampling rate of 1 kHz. The ECG parameters including heart rate, P wave amplitude (mV), PR interval (ms), QRS duration (ms), QT interval (ms), frontal QRS axis (ÂQRS), QR + S amplitude index and ST segment were analyzed using the Chart7 software (PowerLab). Mice with evident alterations in the ST segment were considered in the study. Baseline and post-MI ECG plots are shown in Fig. 2.

2.5.3. *Intramyocardial delivery of liposomes in infarcted myocardium*

Twenty-four hours after the last dose of IPN, cardiac delivery of fluorescent liposomes incorporated in hydrogel was performed by ultrasound-guided transthoracic injection (Vevo 770, VisualSonics, Toronto, Canada). For validation purposes, injection of Evan's blue dye was performed before liposome administration to visualize the local injection in epicardium. Adequate sedation and ventilation of animals was performed (0.5–2% isoflurane, 0.5–0.8 L/min 100% oxygen) and they were placed onto the animal platform. Next, syringes with 29G needles were loaded with liposomes for transthoracic injection guided by a transducer.

Animals were divided in the following groups for receiving liposomes with different sizes (Rhod-Lipo$_{nm}$): Rhod-Lipo$_{165}$ (n = 3), Rhod-

Table 1
Physicochemical characterization of liposomes.

Liposome formulation	Parameters for sonication		Physicochemical parameters		
	Power (W)	Time (sec)	Size ± SD (nm)	PDI	ζ ± SD (mV)
Rhod-Lipo$_{165}$	5	150	165 ± 1	0.289	−8.47 ± 0.6
Rhod-Lipo$_{468}$	5	20–30	468 ± 16	0.323	−9.06 ± 1.3
Rhod-Lipo$_{1551}$	1	50	1551 ± 26	0.515	−2.70 ± 0.3
Rhod-Lipo$_{1954}$	1	10	1954 ± 60	0.643	−1.09 ± 0.2

Rhod-Lipo$_{165,\ 468,\ 1551,\ \text{and}\ 1954}$: rhodamine-loaded liposomes with different mean sizes (nm). SD: standard deviation; ζ: zeta potential; mV: millivolts. PDI: Polydispersity index (PDI).

Lipo$_{468}$ (n = 3), Rhod-Lipo$_{1551}$ (n = 3), Rhod-Lipo$_{1954}$ (n = 3) or only hydrogel (n = 2). A control group of infarcted animals without treatment (n = 2) was also added in this study. All groups received the same dose of liposome (60 mM) and hydrogel (0.4%) in a total volume of 50 μl per mouse. Mice were euthanized after anesthesia with 5% ketamine (Vetanarcol, Konig, Brazil) and 2% xylazine (Sedomin, Konig), 72 h after injection of liposomes or hydrogel. Before being harvested, the hearts of animals were perfused with PBS (5 min) and 10% paraformaldehyde (15 min), respectively. After perfusion, the hearts were harvested and maintained in 10% paraformaldehyde at 4 °C for 24 h. Next, hearts were sliced in three 4-mm-thick segments from apex to base and dehydrated with 70% ethanol at 4 °C for 24 h before histological analysis.

2.5.4. Histological assessment for tissue response and retention of liposomes

Heart slices were embedded in paraffin, cut into sections of 6 μm, and stained with hematoxylin–eosin (HE) and picrosirius red (PSR) to visualize tissue structure, potential inflammation and fibrosis, by optical microscopy. Images were digitized using a color digital video camera (CoolSnap, Photometrics, Montreal, QC, Canada) adapted to a BX41

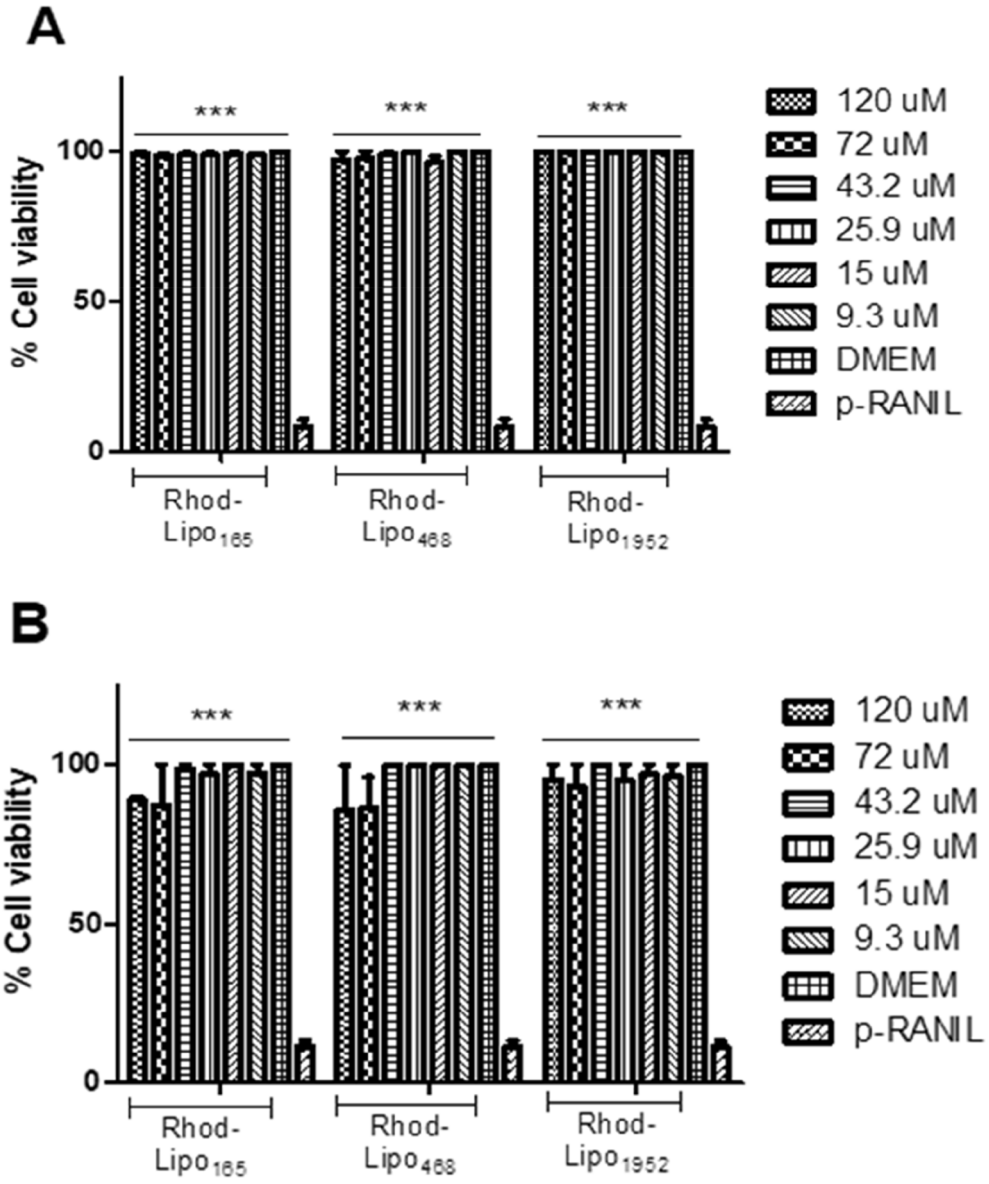

Fig. 3. Cytocompatibility assays of Rhodamine-loaded liposomes with different sizes in L929 fibroblasts (A) and H9c2 cardiomyocytes (B) at different lipid concentrations by the Alamar Blue cell viability reagent. DMEM medium was used as cell viability positive control and pararosaniline chloride (p-RANIL, 10 μM) served as the negative control. ***$p < 0.0001$ vs. p-RANIL.

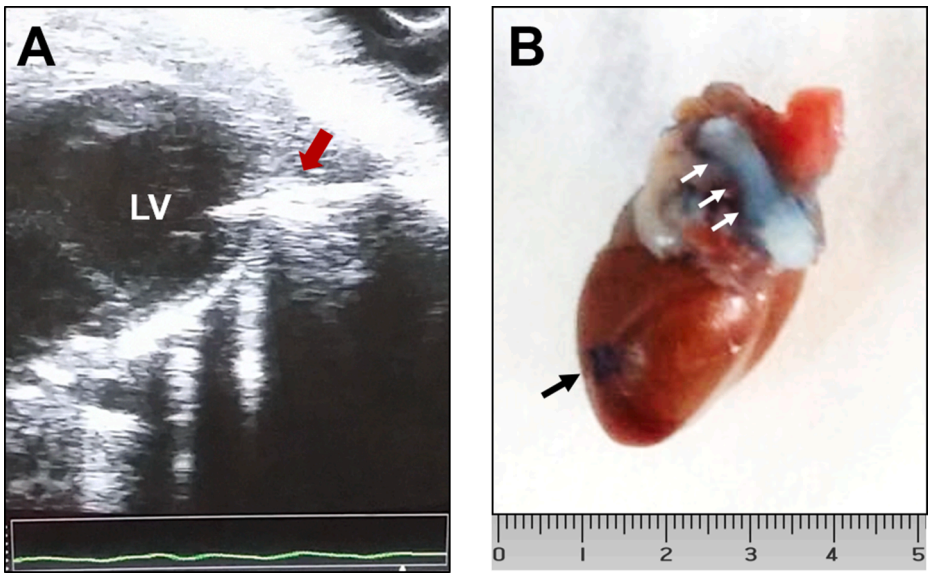

Fig. 4. Ultrasound-guided transthoracic injection of liposomes in ISO-infarcted mouse heart. LV short-axis echocardiography view of injected myocardium with needle reaching the anterior myocardial wall (A, red arrow). Injection point at epicardium (B, black arrow) and tissue distribution of Evan's blue dye towards aorta (B, white arrows). LV: left ventricle; scale in cm.

microscope (Olympus, Tokyo, Japan). Morphometric analyses were performed using the software Image-Pro Plus v.7.0 (Media Cybernetics¸ San Diego, CA, USA). Ten fields (100 μm/field) of each heart were evaluated (40 × magnification), and the total sum of cells in the ten fields represented the total cells/mm^2. The retention of liposomes in the myocardium tissue was evaluated using fluorescence microscopy. All microscopic analyses were carried out as a blind test.

2.6. Statistical analysis

Data are presented as mean ± S.D. Statistical analysis of data was performed with Prism 5.0 software (Graphpad Software Inc., San Diego, CA, USA). For cytotoxicity and *in vivo* assays, differences were assessed by ANOVA with a Tukey post-hoc correction. Shapiro–Wilk test was used to justify the use of a parametric test. A value of $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Liposome characterization

Aiming to assess the optimal liposome size for retention in the heart tissue, liposomes with different sizes were prepared and characterized according to their mean diameter, PDI, and surface charge (Table 1). Changes in acoustic power (W) and time (sec) were applied in the step of sonication of liposome preparation. Smallest liposomes were obtained with sonication for 150 sec at 5 W, resulting in 165 ± 1 nm and PDI of 0.289. At the same power but decreasing the time of sonication (20–30 sec), a 3-fold increase in the size of liposomes was found, 468 ± 16 nm and PDI of 0.323. On the other hand, using the conditions of 1 W power and 10 or 50 secs, larger-sized liposomes were obtained, 1954 ± 60 nm and 1551 ± 26 nm, respectively. These increases were also reflected in increments on PDI, reaching values of 0.515 and 0.643, respectively. Indeed, changes in particle size distribution may be observed when different sonication times are employed [28,29]. With regard to zeta potential, liposomes presented values ranging from − 1.09 ± 0.2 to − 8.47 ± 0.6 mV. Interestingly, changes in sonication conditions seemed do not significantly affect the surface charge of the liposomes, which came close to neutral values as the liposomal size increased.

3.2. Cytotoxicity of liposomes

The effect of different sized-liposomes (165, 468 and 1954 nm) on viability of murine L929 fibroblasts and H9c2 cardiomyocytes was evaluated. As shown in Fig. 3, liposomes did not alter the viability of both cell lines at the lipid concentrations ranging from 9.3 to 120 μM. Indeed, in comparison to the positive control for cell viability (DMEM medium), both cell lines remained at 100% of viability after add of liposomes concentrations. On the other hand, a significant difference ($p < 0.0001$) in the cell viability was observed between liposomes in all concentrations, which kept in 100%, and negative control (p-RANIL), which presented only ~ 20% of viability, indicating a harmful cell effect as expect. Similarly, previous studies evaluated the cytotoxicity of stealthy (0.1–1 mg/ml, 100 nm) and conventional liposomes (10–300 μg/ml, 80 nm) on L929 and H9c2 cells, respectively, and no significant cytotoxicity was found [29,30,31]. In these works, stealthy liposomes consisted of DPPC, cholesterol, PEG-2000 at a composition of 80:20:5 mol%, while conventional liposomes were prepared at the concentration of 10 mg/mL at a 70:30 ratio molar of PC:cholesterol.

Still in the present study, we also assessed the cytotoxicity of the liposome vehicle, a cellulose-based hydrogel as described in material and method section, on H9c2 and L929 cells. As well as observed in liposomes treatment, their vehicle likewise presented 100% of cell viability at concentrations of 5.3 μg/ml – 170 μg/ml for both cells lines (data not shown). These results are in agreement with that previously reported [32], in which no cytotoxicity of bacterial cellulose hydrogel evaluated on C3A or HepG2 human hepatoma cell lines was found. Collectively, these findings ensure that the cellulose-based hydrogel used as an injectable vehicle for the liposomes and the different sized-liposomes are nontoxic materials [33].

3.3. Tissue response and retention of liposomes in infarcted myocardium

The induction of experimental myocardial damage has been previously performed through surgical procedures such as aortic attachment and coronary artery ligation [34]. Although the animal models of MI following coronary artery ligation reproduce many aspects of ischemic cardiomyopathy and heart failure in humans, they suffer from high early mortality and low rate of success of MI [35]. Alternatively, the administration of high doses of isoproterenol, a synthetic catecholamine,

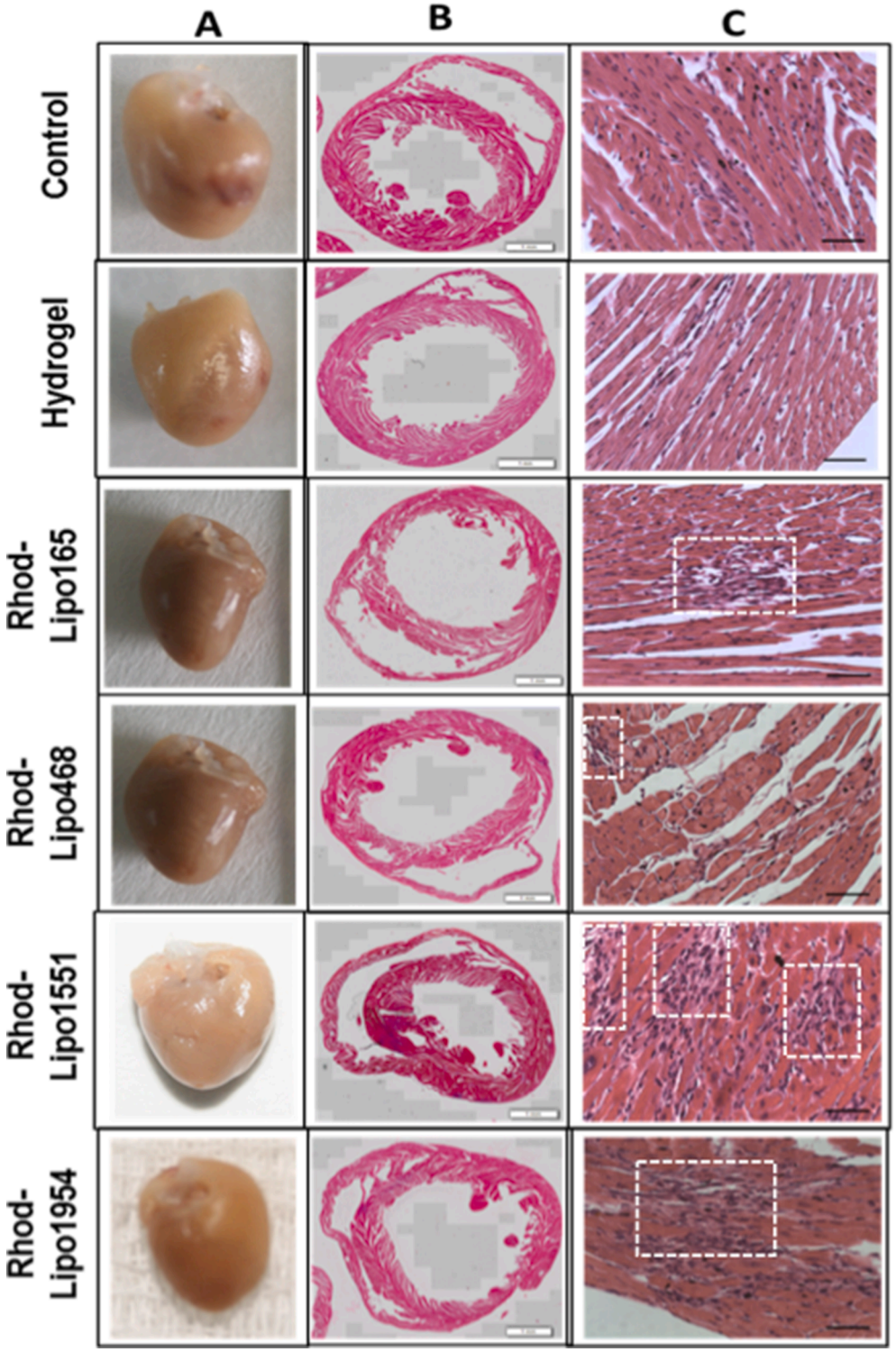

Fig. 5. (A) Macroscopic view of hearts 3 days post-injection of hydrogel and liposomes with different mean size diameter (165, 468, 1551 and 1954 nm) and control. (B, C). Representative photomicrographs of H&E-stained heart sections for tissue structure and inflammation assessment, respectively. White bars: 1 mm; black bars: 50 μm.

provides deleterious histological and biochemical changes in the heart tissue. These effects make for a suitable infarct model since it recapitulates much of the pathophysiology of left ventricular (LV) dysfunction after MI in humans [36,37]. In the present study, mice treated with isoproterenol showed a significant ST elevation in ECG, an indicative finding of MI. No deaths were related during isoproterenol administration and MI was properly induced in mice using a non-invasive and reproducible method.

Emerging therapies for cardiac regeneration using biopharmaceuticals, genes or stem cells face on the critical issue of myocardial delivery. The intramyocardial route (IM) is feasible and suitable for tissue retention [21], but it is commonly performed by invasive methods such as thoracotomy or sternotomy. Conversely, ultrasound-guided percutaneous administration is an alternative and

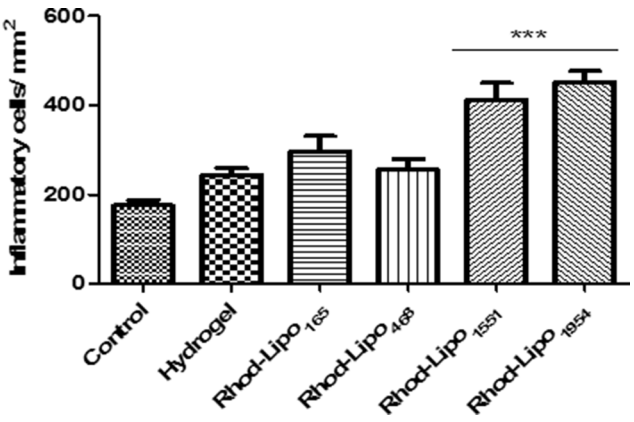

Fig. 6. Analysis of total inflammatory cell infiltration on heart tissue quantified on 10 fields/heart (40 × magnification). ***p < 0.001 vs. control.

feasible method for reaching certain regions of myocardium [23].

This is the first report describing an ultrasound-guided injection of liposomes in infarcted mice. Prior to liposome injection, the procedure was validated by transthoracic injection of Evan's blue dye as previously reported [23]. Thus, it was possible to identify the injection point at the epicardium zone and the tissue distribution of Evan's blue dye content towards LV and aorta (Fig. 4-B). After ensure the efficacy of the procedure, it was possible to perform a real-time visualized liposome delivery on heart tissue in a minimally invasive manner (Fig. 4-A). Importantly, this strategy allowed a safe administration of small liposomes with no cardiovascular damage or injury in other organs. All animals survived to the end of the study. These findings support the ultrasound-guided transthoracic intramyocardial injection as a well tolerated, efficient and minimally invasive procedure with potential for cardiac drug delivery.

Previous studies established that the particle size, the presence of hydrophilic polymer and targeting moieties on surface of liposomes can influence their retention in heart tissue after systemic administration [38,39]. But, despite of relevance of these parameters on biological effects of liposomes, a few number of studies has been focused on their retention and tissue response in animal models of MI [19,38–40]. In this work, after liposome administration, zones with inflammatory infiltrates at different levels were observed in heart mice as function of particle size of injected liposomes (Fig. 5 and Fig. 6). Inflammatory cells were quantified in heart sections and no significant differences were found between hydrogel vehicle, Rhod-Lipo$_{165}$ and Rhod-Lipo$_{468}$ groups compared with the control group. On the other hand, the administration of large liposomes (Rhod-Lipo$_{1551}$, Rhod-Lipo$_{1954}$) showed a statistically significant increase of inflammation ($p < 0.0001$). Although a consistent assessment of the effect of particle size on inducing inflammation of cardiac tissue has not been performed, large particles seem to induce more consistent accumulation of inflammation-mediated cells. Corroborating with this, a previous study investigating PLGA microparticles showed increasing inflammation after intramyocardial injection of 2–30 μm particles [27]. For all liposome sizes, negligible fibrosis was visualized in tissue sections by PSR staining (not shown). Therefore, the levels of inflammation induced by liposomes with 165 nm or 468 nm make them suitable for a safe intramyocardial delivery.

Heart retention of rhodamine-labeled liposomes was confirmed by fluorescence microscopy, indicating their ability to remain in the myocardium for 72 h. Red fluorescence with greater intensity was observed on heart tissue injected with 468 nm-sized liposomes (Fig. 7). In the light of these findings, a 468 nm vesicle size could probably favor positive interactions between liposomes and the cellulose hydrogel, resulting in enhanced tissue adhesion of liposomes. Another factor could be related to macrophage activity of cardiac tissue, affecting the retention of liposomes with different sizes. Correlating with literature, Allijin and colleagues showed a retention of PEGylated liposomes (110 nm, i.v.) at 3 days after MI in mice, but a partial uptake of liposomes by macrophages was detected [41]. In our study, the smallest liposomes (165 nm) achieved only a marginal retention in the myocardium, suggesting an uptake by local macrophages or even a washout due to hemodynamic activity of cardiac tissue. On the other hand, Dvir and colleagues [42] reported an accumulation of labeled PEGylated and targeted-liposomes

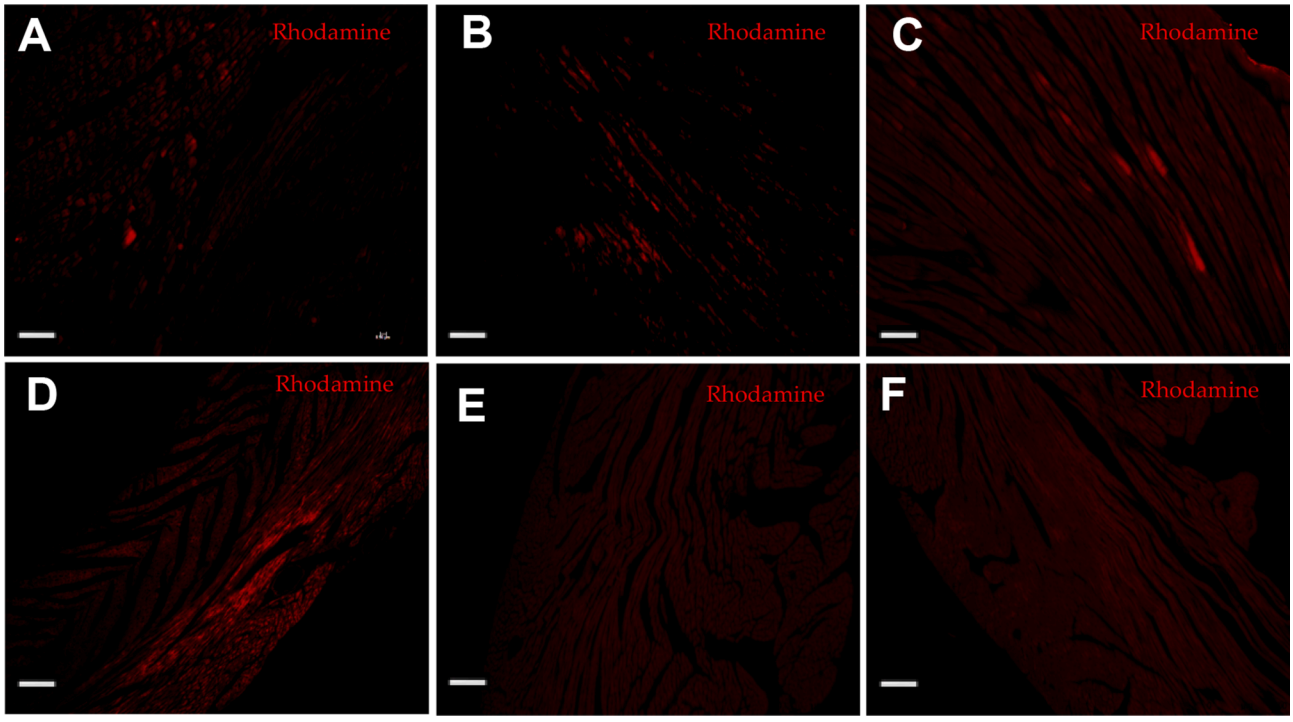

Fig. 7. Myocardium retention of rhodamine-labeled liposomes of various sizes: 165 nm (A), 468 nm (B), 1551 nm (C), and 1954 nm (D) by fluorescence microscopy; hydrogel (E), and untreated control mice (F). Scale bars = 200 μm (20 × magnification).

to angiotensin II receptor (142 ± 8 nm) after 24 h post i.v. injection in infarcted mice.

Recent reports have shown the potential of liposomes for myocardial drug delivery, highlighting their ability for encapsulating therapeutic cargos for cardiac repair. Takase and colleagues developed liposome-encapsulated hemoglobin vesicles as a blood substitute with oxygen-carrying capacity. This liposomal system was able to rescue rats undergoing progressive lethal hemorrhage as a result of an anti-arrhythmogenic effect on the myocardium [43]. In other strategy, liposomes loaded with antigens and rapamycin promoted effective induction of dendritic cells and antigen-specific regulatory T cells. When injected in mice, these liposomes attenuated inflammation in the myocardium and inhibited adverse cardiac remodeling, improving cardiac function [44].

Other carrier systems for delivering biopharmaceuticals to the myocardium have been investigated, such as PEG-modified polystyrene nanoparticles. Upon their administration by tail vein in mice, 200–500 nm diameter nanoparticles were retained by the heart following ischemia–reperfusion, but<1% was retained by cardiac tissue (https://doi.org/10.1038/srep25613). In turn, studies dealing with polymeric micro- and nanoparticles have been conducted [16,17,22,27,45]. For example, 5 μm-sized poly(lactic-co-glycolic acid) (PLGA) particles were compatible with an intramyocardial administration in a rat model of cardiac acute ischemia–reperfusion [27]. In addition, PLGA nanoparticles (190 ± 49 nm) were administered by a minimally invasive procedure in the pericardial space in rabbits, reaching prolonged retention (half-life of ~ 2.5 days) in the entire heart [22]. This is a promising technique for cardiac drug delivery, avoiding surgery and therefore amplifying the clinical translational potential. Similarly, these attributes were also offered by the ultrasound-guided transthoracic injection of liposomes in our study, which demonstrated a longer retention time (3 days).

4. Conclusion

Regarding the limitations of current methods for myocardial drug delivery, this study demonstrated the feasibility of cardiac delivery of liposomes in a convenient and non-surgical manner using transthoracic injection and echocardiography. Within this work, the *in vitro* compatibility, tissue retention and myocardial safety of 468 nm-sized liposomes were featured. These findings indicate the ability of liposomes for reaching efficiently the myocardium via ultrasound-guided injection. This work warrants attention for further research, including biodistribution studies with drug-containing liposomes to better understand their potential in treatment of CVD.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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