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Biodegradable dexamethasone polymer capsule for long-term release

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Abstract−We have developed sustained Dex (dexamethasone) capsule implants for sustained local delivery for inflammatory disease treatment. Four different biodegradable polymers were used as capsule materials: polycaprolactone (PCL), poly(lactic acid) (PLA), 90 : 10 poly(lactic-co-glycolide) (PLGA), and 50 : 50 PLGA. The drug release profiles from the four types of capsule were compared and the profiles were fit to a cylindrical reservoir first-order kinetics model. As a result, 50 : 50 PLGA showed the fastest release with the largest permeability and partition coefficient at 0.4909 nm/s and 1.9519, respectively. On the other hand, PCL showed the slowest release with the smallest permeability and partition coefficient at 0.1915 nm/s and 0.8872, respectively. The results indicate that the drug release kinetics are highly correlated with hydrophobicity of the polymer sheet: the more hydrophobic, the slower the drug release kinetics for the hydrophilic drug. The in vitro therapeutic efficacy of the Dex implant was also explored using TNF-α stimulated human umbilical vein endothelial cells (HUVECs), showing effective suppression of IL-6 levels with the implant compared to free Dex with minimal toxicity. Overall, this study suggests that the release trend of Dex from implants follows the hydrophobicity of each polymer, and the Dex implant inhibits the IL-6 expression effectively.

Keywords: Long-term Drug Delivery, Injectable Implant, Biodegradable, Dexamethasone, PLGA, PCL, PLA

INTRODUCTION

Dexamethasone, one of the most widely used corticosteroids, is an effective anti-inflammatory drug commonly prescribed for chronic inflammatory diseases, such as asthma, allergic rhinitis, urticaria, and many other conditions. Dexamethasone (Dex) has been also employed to prevent and/or reduce vocal fold scarring because of the effect on wound healing, improving post operative voice quality in patients undergoing phonosurgery [1]. However, the use of Dex is limited by dosing. Oral dosing is less effective in target tissues because of the necessary high dose and is limited by serious side effects, including ocular, musculoskeletal, and dermatologic diseases [2,3]. In addition is the need for frequent injections for local delivery in places challenging to access, such as the larynx. Local injections to the vocal folds are limited by the complex three-dimensional laryngeal anatomy and lack of easy access.

Due to the short half-life of Dex being 5.5 h, many studies have been focused on exploring a drug delivery system for its long-term sustained release. One successful example is the intravitreal Dex implant (Ozurdex, Allergan Inc, Irvine, CA), which is a rod-shaped implant made of solid biodegradable poly(lactide-co-glycolide) (PLGA) polymer. This Dex implant is designed to release 700 μg of Dex over six months with a peak concentration at day 22, and was approved by the Food and Drug Administration (FDA) for the treatment of macular edema in 2009 with minimum side effects [4]. However, most of the drug is released within 1-2 months [5-7]. PLGA used in Ozurdex is a polyester-based polymer, which is the most widely investigated for drug delivery. Other polyester-based polymers include polycaprolactone (PCL), poly(lactic acid) (PLA), and poly(glycolic acid) (PGA). Many drug delivery systems have been explored based on design and synthesis of combinations of these polymers [8,9].

Several studies demonstrated therapeutic delivery of growth factors or extracellular matrix (ECM) compositions to the vocal fold utilizing biocompatible polymers such as PCL, with no significant immune or foreign body responses [10-13]. In addition, one study has shown that a surgical micro-clip made of magnesium for laryngeal microsurgery suppressed inflammation when the surface was coated with PCL [14]. These studies indicate that biocompatible polymers are very promising tools for the delivery of desired treatment in the vocal fold.

However, it is nontrivial to select a drug delivery carrier among the polymer options because of miscibility between a drug and a polymer, a drug release kinetics, and safety against immune responses and toxicity [9]. In this article, four different types of polymers with different compositions, PCL, PLA, 90 : 10 PLGA, and 50 : 50 PLGA, were used as a drug delivery capsule implant and tested for release of dexamethasone. All of the selected polymers are aliphatic polyesters, and also FDA-approved in many medical applications.

PCL is a semicrystalline linear polyester obtained by the ring-opening polymerization of epsilon-caprolactone. PLA is usually produced through the direct condensation reaction of its monomer lactide and is a hydrophobic polymer due to the CH_3 side groups. PGA is prepared by ring opening polymerization of a cyclic lactone, glycolide. Due to its excellent mechanical properties but low solubility and high degradation rate against acidic products, PGA has

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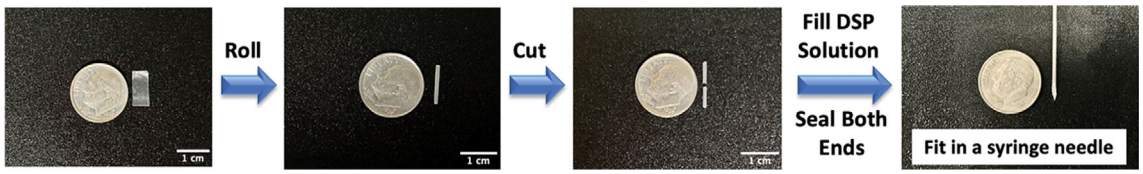

Fig. 1. Implant fabrication process.

always been prepared as copolymers. PLGA is a copolymer of PLA and PGA and different ratios of PLGA have been commercially developed. When the ratio of lactide/glycolide (L/G) increases, the degradation rate of the copolymer decreases and the hydrophobicity increases [15].

The objective of this study was to investigate how Dex is released from four different types of polymers and to confirm the feasibility of the mathematical models using permeability and partition coefficient for the drug release profile. In this study, dexamethasone sodium phosphate, a water-soluble salt form of Dex, was used. Because of the high water-solubility, it allows the administration of relatively high doses in a small volume of aqueous diluent for drug injection. The in vitro delivery efficiency for anti-inflammatory effects and the cytotoxicity of the implant were also tested using TNF-α stimulated human umbilical vein endothelial cell (HUVECs).

MATERIALS AND METHODS

1. Materials

Poly(caprolactone), PCL (MW 65,000-75,000), Poly(L-lactide), PLA (MW 100,000-125,000), Poly(lactide-co-glycolide), PLGA 90 : 10 (L : G 90 : 10, MW 100,000-200,000), Poly(lactide-co-glycolide), PLGA 50 : 50 (L : G 50 : 50, MW 15,000-25,000) were purchased from PolyScitech, Inc (West Lafayette, IN). Dichloromethane (DCM), potassium chloride (KCl), and MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) were purchased from Fisher Chemical (Waltham, MA). Dexamethasone sodium phosphate powder was purchased from The Lab Depot (Dawsonville, GA). Human umbilical vein endothelial cell (HUVECs), EBM™-2 Basal Medium and EGM™-2 SingleQuots™ Supplements were purchased from Lonza (Basel, Switzerland). Human IL-6 Matched Antibody Pair Kit and ELISA Accessory Pack were purchased from Abcam (Cambridge, UK). Corning UV-transparent microplate was purchased from Sigma Aldrich (St. Louis, MO).

2. Synthesis of Polymer Sheets

A polymer solution in DCM at 50 mg/mL was transferred into a mold (1,500 μL) that was created by attaching two stainless-steel rectangles (4.9 cm×3.1 cm×1.5 cm, length×width×height) to a phone glass protector using superglue. DCM was slowly evaporated with a cover on at 15 °C overnight to create the polymer sheet. The dry sheet was then removed by a razor blade.

3. Implant Fabrication

The polymer sheet was cut into 1 cm×0.5 cm pieces using a razor blade for implant fabrication. The pieces were then rolled around a 22-gauge needle at 45 °C to create a double layered tube with 1 cm in length and 0.0946 cm in diameter. Two implant tubes with 0.5 cm in length were created by cutting the 1 cm implant tube into half. One end of the tube was clamped with a 60 °C iron. A 26-gauge needle was used to load 1.3 μL of the 500 μg/μL Dex solution into the tube, resulting in 650 μg encapsulation. Once the Dex solution was loaded, the iron was used to seal the other end and then both ends were superglued to prevent any possible leakage. The implant fabrication process is shown in Fig. 1, and the dimension of the final implant is ~0.9 mm in diameter and 5 mm in length. As shown in the figure, the implant was placed inside an 18-gauge syringe needle with sealed ends. The final dimension of the implant is 5 mm in length and 0.464 mm in outer diameter.

4. Drug Release Profile

Implants loaded with the Dex solution were placed in a test tube with 1 mL of PBS and incubated at 37 °C. For each polymer, four implants were made. The PBS solution was removed and replaced with fresh PBS every 24 h. The removed solution was then analyzed using UV-Vis at 240 nm to determine the Dex concentration. Using the concentration with calibration curve, the amount of Dex released was plotted to determine the release profile for a time period of 42 days.

5. SEM Imaging

The surface and cross-section structures were analyzed using scanning electron microscopy (SEM) (Thermo Fisher Scios Dual Beam SEM, Hillsboro, OR). The polymer sheets were attached to horizontal or vertical SEM sample holders using double sided graphite tape. The samples were sputter-coated with 5 nm platinum/gold nanoparticles for 10 s using Denton Vacuum Desk II (Moorestown, NJ). 1 cm×0.5 cm sheets were coated in glycerol, submerged in liquid nitrogen, then fractured to obtain a clean cut to view the cross-section. Fractured sheets were then rinsed with DI water, dried overnight, and placed on vertical sample holders to image the cross-section. The thickness of the sheets was analyzed using ImageJ (NIH).

6. Porosity Measurement

The porosity of the polymer sheets was determined by using a setup of a humidity chamber with potassium chloride (KCl) as the saturated salt solution. A synthesized polymer sheet was cut into two 0.5 cm×0.5 cm pieces with a razor blade. A double layered polymer sheet was then created by placing the two pieces on top of each other and pressing them together at 45 °C. Three double layered polymer sheets were made for triplicates. All dry weights of the double layered polymer sheets were recorded before taking inside the chamber. A weight boat filled with KCl was placed inside the petri dish. Once the double layered polymer sheets were moved into the humidity chamber as shown, DI water was added to create a saturated KCl salt solution. The top of the petri dish was covered and wrapped with parafilm to prevent any possible leakage of the vapor. After 48 h, wet weights of the double layered polymer sheets were measured.

Porosity was calculated using the following equation:

$$\varepsilon = \left[\frac{(V_w - V_d)}{V_w}\right] * \frac{1}{RH} \quad (1)$$

where V_d is the volume calculated from the dry weight and the density of the polymer, V_w is the volume calculated from the wet weight considering the density of water and the polymer, and RH is the relative humidity of the saturated salt solution. In this study, the relative humidity of KCl, salt solution used, is 85% at 25 °C [16].

7. Permeability Measurement

Vertical Franz diffusion cells were used to conduct the permeability experiments. The donor side was filled with 200 μL of 30 mg/mL Dex solution and the receiver side was filled with 5 mL of PBS. The concentration of the donor solution was estimated based on the release profile. A 1.3 cm×1.3 cm double layered polymer sheet was used as the membrane. To measure the permeability, a 1.3 cm× 1.3 cm double-layered polymer membrane was made in a similar way to the membranes in the porosity experiment. Once the membrane was securely placed between the donor and receiver chambers, 200 μL of the PBS solution was loaded for a one-hour equilibration. After equilibration, the PBS solution was exchanged by the donor solution and then the opening was sealed with parafilm to reduce evaporation altering the concentration of the donor and receiver solutions. 300 μL of the receiver solution and 10 μL of the donor solution were removed every 24 h to determine the Dex concentration using UV-Vis. Fresh PBS was used to replace the removed receiver solution, and the donor side was also replaced by fresh donor solution. Depending on the remaining concentration of the Dex donor solution, fresh 30 mg/mL Dex solution might be used to replace the solution in the donor side.

After four days, the mass of Dex that permeated the membrane was plotted against time to get the release over time dQ/dt in the equation:

$$C_i \cdot P = \frac{1}{A} \cdot \frac{dQ}{dt} \quad (2)$$

where C_i is the initial concentration of the Dex donor solution, P is the permeability coefficient of the membrane, Q is the amount of Dex permeated at time t, and A is the area of the exposed membrane.

8. Partition Coefficient Measurement

Partition coefficient K was determined for the model equation used to predict the release kinetics. A 0.5 cm×0.5 cm double-layered polymer membrane was made in a similar way to the membranes in the porosity experiment to measure the partition coefficient. The membranes were soaked in 300 μL of PBS for 48 h. After 48 h the membranes were tipped on a Kimwipe to remove the excess surface liquid and then weighed to determine the wet mass of the membrane. The membranes were then dried overnight in a chemical hood. The dry membranes were then placed in 300 μL equilibrium Dex solution at a concentration of 11.95 mg/mL for 72 h for the membranes to absorb the Dex from the solution. After 72 h, the membranes were removed from the Dex solution. Kimwipe was used again to get rid of the excess solution on the membranes and then the membranes were placed in 300 μL of fresh PBS to extract Dex for 48 h. This extraction process was repeated until the amount extracted was at least 90% less than the first extraction based on the UV-Vis. The remaining equilibrium solution and all extraction solutions were analyzed using UV-Vis to determine the Dex concentration. The partition coefficient was then determined using the equation below:

$$K = \frac{\frac{M_e}{W}}{\frac{M_{eq}}{V * \rho}} \quad (3)$$

where M_e is the total mass extracted, W is the wet mass of the membrane, M_{eq} is the amount of Dex left in the equilibrium solution, V is the volume of equilibrium solution left, and ρ is the density of the solution [17].

9. Modeling Equations

The modeling equation [18] used for predicting the release profile is

$$\frac{M_t}{M_\infty} = 1 - \exp\left[-\frac{(R_i L + R_o L + 2R_i R_o) D_e K t}{R_i^2 L (R_o - R_i)}\right] \quad (4)$$

where M_t is the mass of Dex released at time t, M_∞ is the mass of Dex loaded in the implant, R_i is the inner radius of the implant, R_o is outer radius of the implant, D_e is the effective diffusivity, K is the partition coefficient, and L is the length of the implant. This modeling equation follows Fick's law of diffusion of molecules from the lumen of a cylindrical implant through a membrane wall. Variables, R_o, R_i, and L, were measured from the implants directly. Partition coefficient K was determined experimentally as described in section 2.8.

D_e was determined by the following equation, assuming the Dex molecules diffuse through the pore spaces of porous media [19]:

$$D_e = \frac{\varepsilon D}{\tau} \cdot K_r \quad (5)$$

where ε is the porosity of the membrane, D is the Stokes-Einstein diffusion coefficient, τ is tortuosity, and K_r is the restrictive factor when the ratio of the molecule diameter (d_m) and pore diameter (d_p) is less than 1. The restrictive factor K_r between the diameters is expressed by:

$$K_r = \left[1 - \frac{d_m}{d_p}\right]^4 \quad (6)$$

D, the Stokes-Einstein diffusion coefficient was calculated based on Eq. (7):

$$D = \frac{k_B T}{6\pi\eta r} \quad (7)$$

where k_B is the Boltzmann constant, T is the temperature in Kelvin, η is the viscosity of water at 25 °C, and r is the hydrodynamic radius of Dex. The porosity ε was determined experimentally as described in Section 2.6; the ratio of restrictive factor Kr over tortuosity τ of all polymers tested was obtained by fitting the data. The release profile data were fit to Eq. (4) using Excel. The actual release at Day 5 and Day 14 was compared to the model prediction values and the percent error was calculated.

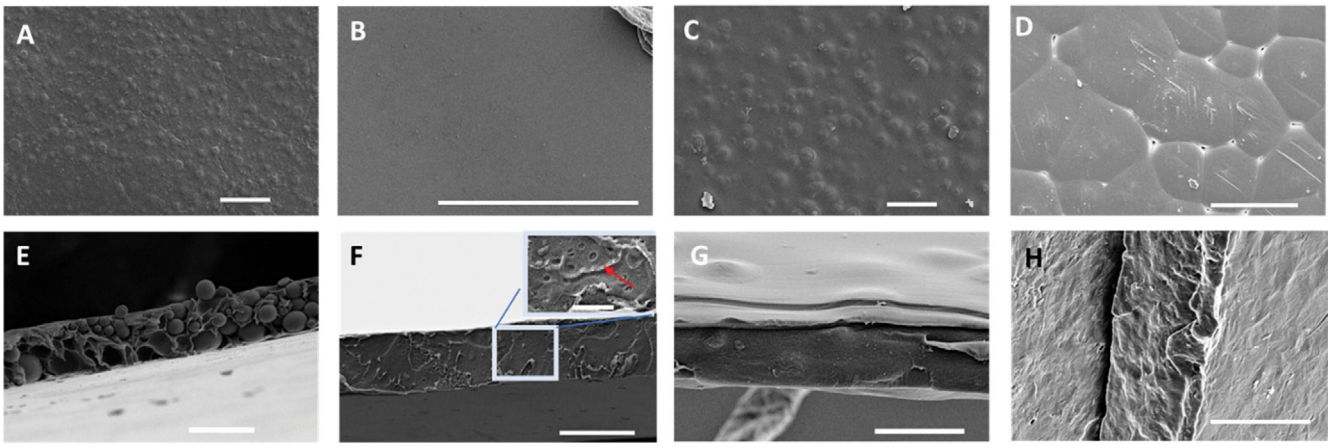

Fig. 2. SEM images of (A, B, C, D) Top sheet of 50 : 50 PLGA, 90 : 10 PLGA, PLA, and PCL, respectively. Scale bars=100 μm. (E, F, G, H) Cross-section of 50 : 50 PLGA, 90 : 10 PLGA, PLA, and PCL, respectively. Scale bars=20 μm (Zoomed image scale bar=5 μm).

10. Cell Culture and Enzyme-Linked Immunosorbent Assay (ELISA)

Human primary umbilical vein endothelial cells (HUVECs) were maintained in EBM™-2 Basal Medium and EGM™-2 SingleQuots™ Supplements (Lonza, Switzerland). HUVECs were grown in the cell incubator containing 5% CO_2 at 37 °C. When the cells were grown to 90% confluence, they were inoculated in 24-well plates. Briefly, 0.4 mL of HUVECs suspensions (8×10^4 HUVECs per well) were seeded in 24-well plates and incubated at 37 °C, 5% CO_2 for 24 h. Experimental conditions (PBS-implant, Dex-implant, and free Dex) were treated with the respective conditions at Dex concentration of 33 μM. After 24 h, all the conditions except the medium only were incubated with 20 ng/mL TNF-α for 24 h. The Dex concentration was selected based on the release kinetics; ~33 μM of Dex was released from the 100 μg Dex-loaded implants within 24 h of incubation. At the end of experiments, the cell medium was collected and centrifuged at 2,000xg for 10 min to remove the particulate/debris materials. Then, supernatant was collected and stored at −20 °C until used. Each sample was pre-diluted using a standard dilution buffer to keep the sample concentrations within the range of standard curve. The concentration of pro-inflammatory cytokine (IL-6) in the cell culture supernatant was analyzed by the ELISA assay according to the manufacturer's recommendation (Invitrogen human IL-6 ELISA kit). All tests were done in triplicate.

11. Cell Viability Assay

The cell viability of HUVECs was determined using an MTT assay. The cytotoxicity of PBS-implant, Dex-implant and free Dex was investigated after 24 h of pretreating HUVECs followed by 20 ng/mL TNF-α treating for the other 24 h. Briefly, MTT reagent (5 mg/mL) in the medium was added to each 24 well plates after the supernatant were collected for the ELISA assay and incubated at 37 °C and 5% CO_2 in the cell incubator for 3 h. Then, the medium was removed and 200 μL of dimethyl sulfoxide (DMSO) was added to each well plate to dissolve the yellow formazan precipitates. After complete homogenization under shaking, 100 μL was collected from each well and added to 96 well plate and the absorbance was measured at 492 nm on a microplate spectrophotometer reader (Spectramax, Molecular Devices, LLC). All experiments were done in triplicate.

RESULTS

1. Characterization of the Polymer Sheets

The surface of 50 : 50 PLGA and PLA shows a round bumpy structure (Figs. 2A and 2C). The 50 : 50 PLGA's round bumps range from 8-20 μm, while the PLA's bump size is consistently around 10-30 μm. 90 : 10 PLGA and PCL does not show the bumpy structure prominently (Figs. 2B and 2D). All four polymers have a smooth bottom surface from being in contact with the mold during synthesis (Fig. S1). The cross-section images of the 50 : 50 PLGA and PLA show a spherical structure (Figs. 2E and 2G), which affects the bumpy structure on the surface in Figs. 2A and 2C, respectively. 90 : 10 PLGA has small pores, between 0.5-1 μm, distributed throughout the cross-sections (Fig. 2F). PCL has a smooth cross-section (Fig. 2H). The spherical and bumpy structures in 50 : 50 PLGA and PLA are most likely caused by the hydrophilicity of the polymers during the evaporation of DCM. We speculate that the more hydrophilic the polymer, the faster the evaporation rate of DCM, causing the structures [20].

PCL has the thickest membrane with a thickness of 20.5±0.37 μm, followed by PLA with a thickness of 17.8±1.11 μm, 50 : 50 PLGA with 16.2±0.42 μm, and 90 : 10 PLGA with 16.3±0.42 μm. The thickness of the two PLGA ratios does not significantly differ, and they both are significantly different from PLA and PCL ($p< 0.05$). PLA and PCL also has a significant difference in their thicknesses ($p=0.025$). The slight decrease in the thickness of the two PLGAs from the PLA may be due to the addition of glycolide. This addition reduces the number of branches from the lactide and reduces thickness slightly.

2. Drug Release Profile from Polymer Capsules

The release profiles from the implants show that almost 90% of Dex was released within ~15 days for the four polymer implants as shown in Fig. 3. Four polymer implants' plateaued at ~600 μg, with ~50 μg of the Dex left in the polymer membrane. The ~50 μg is left in the polymer tube dimension because of thermody-

namic equilibrium, the partition coefficient. Using the partition coefficient value, average K=1.52, and Eq. (3), M_e interpreted as the amount of Dex in the polymer was calculated as 46 μg. Briefly, $M_e = K\left(\frac{M_{eq}}{V*\rho}\right)W$, where $\frac{M_{eq}}{V*\rho}$ was approximated as the solubility of Dex in water at 293.15 K, 0.02168 g/g [20], and W=0.5 cm×0.5 cm×40 μm×1.4 g/cm^3. The release was fast in the first five days, releasing around 80% before slowing to release 20% in the next ten days. As the concentration gradient across the membrane decreases over time, the release rate with time decreases as expected. Based on the release profile, Dex releases slowest from PCL, fastest from 50 : 50 PLGA, probably due to the hydrophobicity, which will be discussed in the next section.

3. Permeability (P), Partition Coefficient (K) and Porosity (ε) of the Polymer Sheets

Fig. 4 shows the cumulative Dex amount that permeated across four different types of polymer membrane over time. The permeation is assumed at steady state and the permeability coefficient is

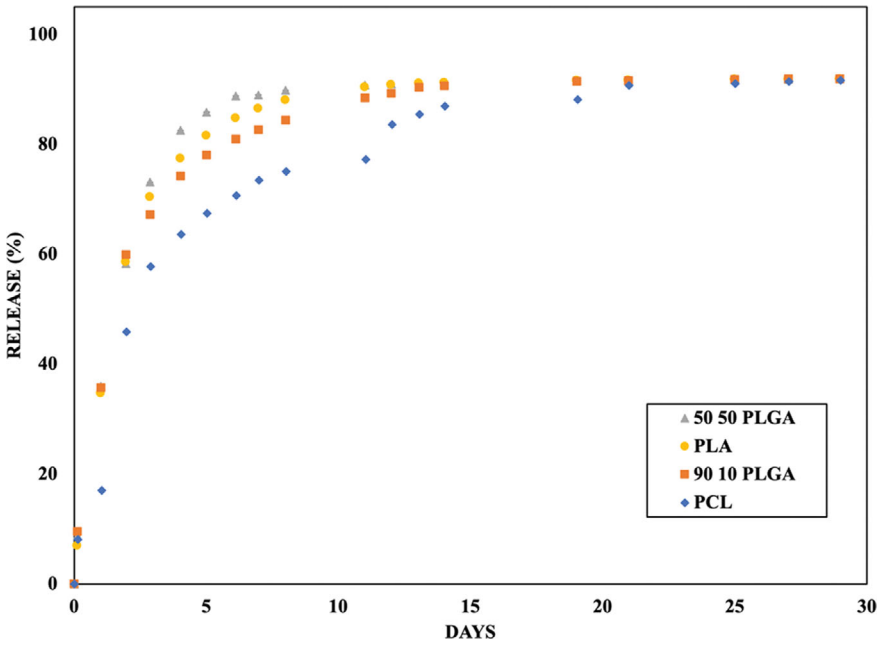

Fig. 3. Dex release profile in percentage vs. time for 50 : 50 PLGA (triangle), PLA (circle), 90 : 10 PLGA (square), and PCL (diamond).

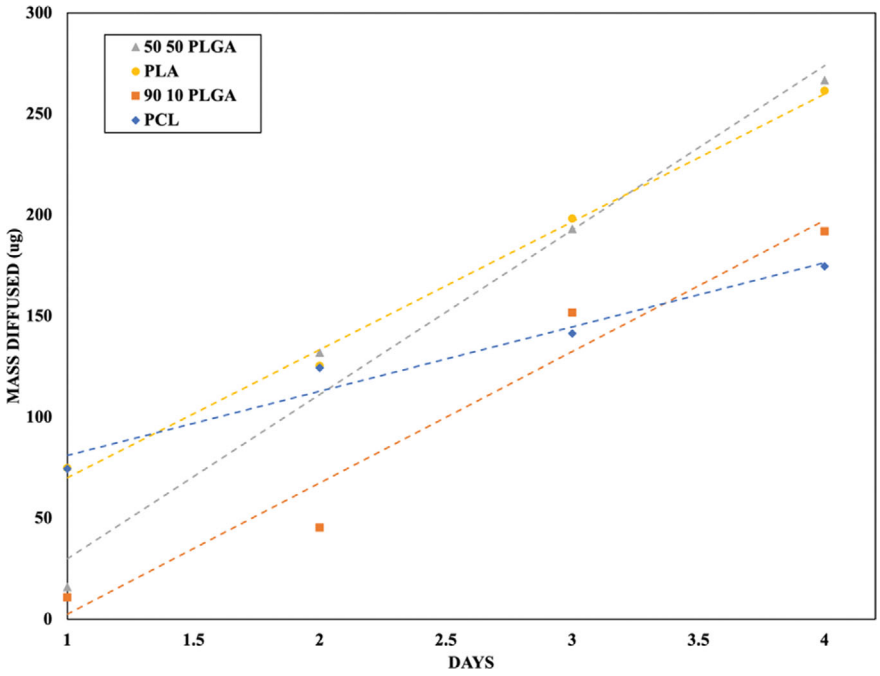

Fig. 4. Cumulative drug permeation vs. time for 50 : 50 PLGA (triangle), PLA (circle), 90 : 10 PLGA (square), and PCL (diamond).

Table 1. Experimental parameters: permeability coefficient, partition coefficient, and porosity for all four polymers tested

Polymer sheets	Permeability coefficient P (nm/s)	Partition coefficient K	Porosity (ε)
50 : 50 PLGA	0.4904	1.9519	0.0707
PLA	0.3816	1.6492	0.0759
90 : 10 PLGA	0.3916	1.6019	0.0986
PCL	0.1915	0.8872	0.0577

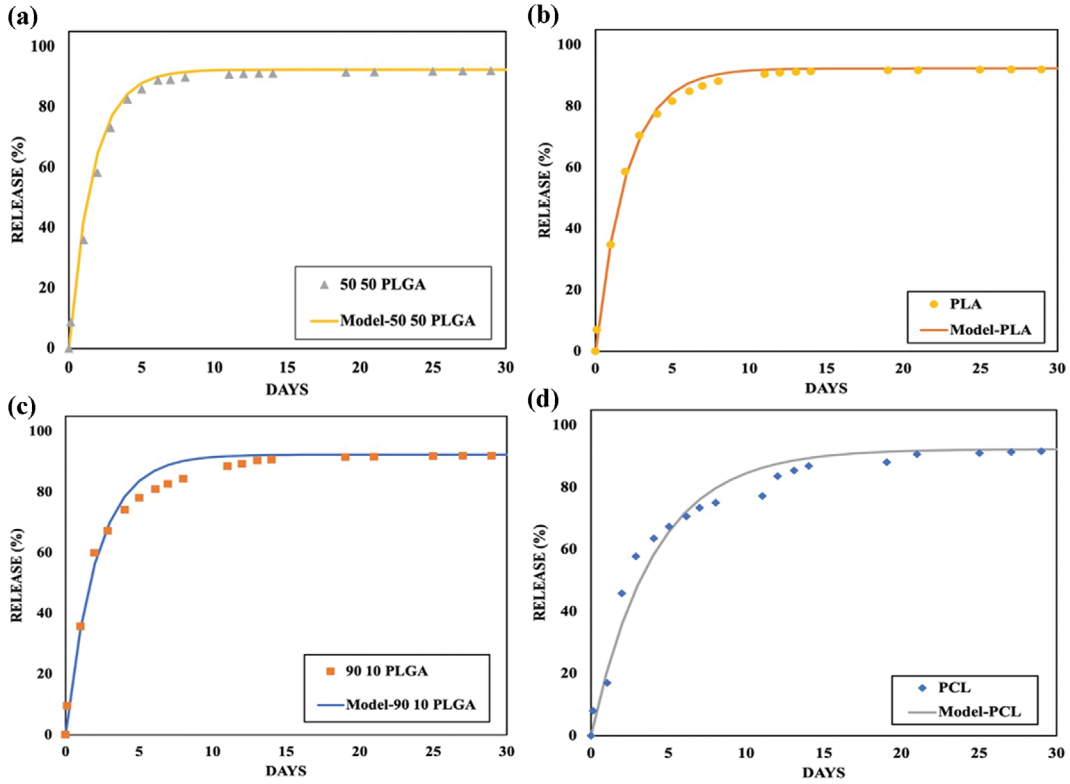

Fig. 5. Model fitting for (a) 50 : 50 PLGA, (b) PLA, (c) 90 : 10 PLGA, (d) PCL of 30 days release profiles with actual data.

determined from the slope of the line using Eq. (2). Based on the slope of the lines, 31.751 μg/day, 63.299 μg/day, 64.96 μg/day, and 81.35 μg/day of Dex permeated across the membrane for PCL, PLA, 90 : 10 PLGA, and 50 : 50 PLGA, respectively. All permeability coefficients are listed in Table 1 for the four polymer sheets. For the partition coefficients, the concentration of equilibrium Dex solution was measured and determined using Eq. (3) (Table 1). A trend between the partition coefficient and permeability coefficient is shown. As the partition coefficient increases, the permeability coefficient also increases, which is also directly related to the hydrophobicity of the polymers. Because Dex tested in this study is hydrophilic, the permeability through the hydrophobic materials is lower than the one through the hydrophilic materials.

The porosity values of the polymer sheets determined experimentally (Table 1) were similar. ANOVA tests were performed among the groups to determine if they are significantly different or not. All of the p-values were greater than 0.05, which means the porosity values among the samples were not statistically different.

4. Drug Release Model

The Dex release profiles were fit to a cylinder reservoir shape model using Eq. (4) for the four polymer implants as shown in Fig. 5. The effective diffusivity was obtained by Eq. (5) by adjusting the ratio of Kr/τ in this modeling, as Kr and τ both depend on the pore diameter, and this will be further addressed in the discussion section. In our case, SEM was not capable of capturing the pore diameter for the polymers. By adjusting Kr/τ and using the experimentally determined values including permeability coefficient and porosity, the models fitted the actual release data relatively well in all four implants. All the values used in the modeling are shown in Tables 1 and 2. At day 5, the actual Dex release for PCL, PLA, 90 : 10 PLGA, and 50 : 50 PLGA was 67.42% (438.23 μg), 81.63% (530.60 μg), 78.03% (507.20 μg), and 85.85% (558.03 μg), respectively, and the model's calculated release was 65.66% (426.79 μg), 84.31% (548.02 μg), 83.61% (543.47 μg), and 87.87% (571.16 μg), respectively. The percent error between the actual and model release was 2.61%, 3.28%, 7.15%, and 2.35%, respectively. At day 14, the actual Dex release for PCL, PLA, 90 : 10 PLGA, and 50 : 50 PLGA was 86.95% (565.18 μg), 91.27% (593.26 μg), 90.59% (588.84 μg), and 91.02% (591.63 μg), respectively, and the model's calculated release was 89.46% (581.49 μg), 92.21% (599.37 μg), 92.18% (599.17

Table 2. Fitting parameters for Kr/τ and D_e from Eq. (5)

Polymer capsules	R_i (cm)	R_o (cm)	L (cm)	Kr/τ	D_e (cm^2/day)
50 : 50 PLGA	0.0413	0.0464	0.5	0.001059	2.84×10^{-5}
PLA				0.000942	2.71×10^{-5}
90 : 10 PLGA				0.000721	2.69×10^{-5}
PCL				0.001169	2.55×10^{-5}

μg), and 92.30% (599.95 μg), respectively. The percent error between the actual and model release was 2.88%, 1.03%, 1.76%, and 1.41%, respectively. The overall trend for the release profile matched for all the polymer implants, indicating that the Dex release profile from the capsules followed the first-order kinetics of a cylindrical reservoir [21]. The Dex release from the PCL was the slowest, implying that the Dex PCL implant would be ideal for a sustained-release over 21 days among the four polymers tested.

5. Dex-implant Reduces TNF-α-induced Inflammation

Because the PCL showed the most sustained- release, we chose the PCL implant to test the drug delivery efficiency in vitro and the cytotoxicity. To Investigate therapeutic effects of the Dex-loaded PCL implant (condition 5) to protect HUVECs from TNF-α-induced inflammation, the levels of pro-inflammatory cytokine, IL-6, were quantified using ELISA assays. As seen in Fig. 6, the ELISA results show that the concentration of IL-6 was significantly increased ($^{\#}p<0.05$) in the TNF-α stimulated HUVECs (condition 2) in comparison to the negative controls (treated only with medium, condition 1), which confirms TNF-α induces HUVECs inflammation. The IL-6 level in the cell culture medium significantly decreased ($^{**}p<0.01$) in the Dex-loaded implants (condition 5) compared to condition 2, indicating the Dex was effectively delivered to the HUVECs to inhibit inflammation. Interestingly, the IL-6 level in condition 4 with the free Dex (condition 4) was not significantly decreased, which may imply that sustained slow release of Dex from implant for 48 h is more effective than single time injection of free Dex, to reduce TNF-α induced HUVECs inflammation. Lastly, the IL-6 level of the PBS-implant (condition 3) was not statistically different from the positive control (condition 2), indicative of no effect of the implant only on the inflammation inhibition.

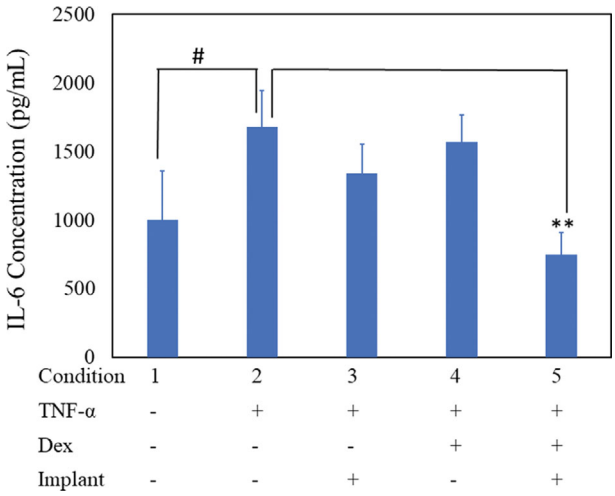

Fig. 6. IL-6-ELISA assay using TNF-α stimulated HUVECs. #p<0.05, **p<0.01.

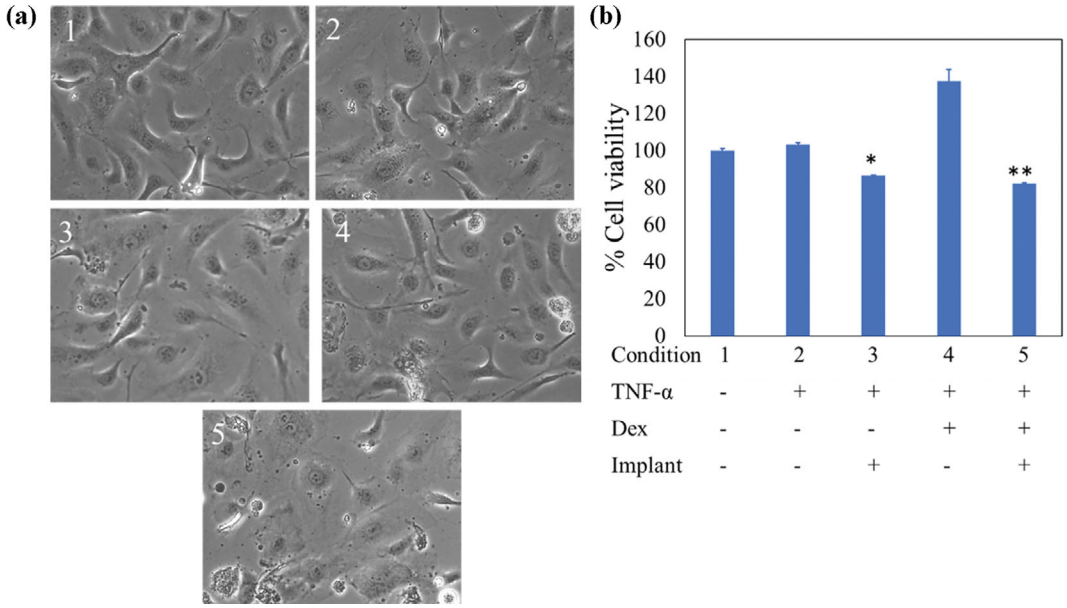

Fig. 7. (a) Representative images of HUVECs for the five conditions before adding MTT reagents. (b) In vitro cytotoxicity results using MTT assay. *p<0.05, **p<0.01 vs. Condition 1.

6. Effects of PBS-implant, free Dex and Dex-loaded Implant on HUVECs Viability

The cytotoxicity effects of PBS-implant (condition 3), free Dex (condition 4) and Dex-loaded implant (condition 4) were investigated via optical imaging and MTT assay using TNF-α stimulated HUVECs. As shown in Fig. 7, more than 80% of HUVECs were alive in all treatment groups, including free Dex, PBS-implant, and Dex-loaded implant, indicating that the Dex with the dose tested and the polymer implant have negligible toxicity to HUVECs. Although the cells in all conditions looked healthy in the microscope images, the MTT assay results show that the PBS implant and Dex-loaded implant (conditions 3 and 5, respectively) had significantly lower cell viability than the negative control (condition 1) with p-values 0.02 and 0.005, respectively.

DISCUSSION

The release profile in Fig. 3 shows that almost 100% Dex was released from the hydrophilic implants in the first 15 days, which followed the first-order release kinetics. The release from PCL was slow at the beginning compared to the other three polymers, whereas the other three polymers had similar release for the first few days. The difference in the release appeared at day 5, in which the 50 : 50 PLGA released fastest compared to the other three.

Dexamethasone sodium phosphate is a hydrophilic drug. Theoretically, as the hydrophobicity of the polymer sheet increases, the slower the Dex releases from the capsules. PCL is a well-known hydrophobic polyester used in many applications. PLGA is a copolymer of poly(lactic acid) (PLA) and poly(glycolic acid) (PGA). Because the methyl side groups in PLA makes it more hydrophobic than PGA, 90 : 10 PLGA is more hydrophobic than 50 : 50 PLGA. PCL is the most hydrophobic, and 50 : 50 PLGA is the most hydrophilic out of the four polymers, which is attributed to the release kinetics.

The permeability coefficient is a quantitative measure of the rate at which molecules can cross a membrane. A high permeability coefficient indicates the rate of the flow is high, which is directly related to the effective diffusion coefficient D_e. The trend of the permeability exactly matched the values of D_e (Table 2). Although D_e described in Eq. (5) is a function of physical properties of the porous media, such as porosity and tortuosity, our results suggest that the Dex diffusion through the media is not solely governed by the physical properties but also the chemical properties of the membrane. This is because the D_e did not exactly follow the trend porosity ε, but followed the partition coefficient. The partition coefficient is a measure of a solute's solubility or distribution in the media. In this study, the greater the partition coefficient, the higher the permeability of the membrane to the solute. 50 : 50 PLGA has the greatest partition coefficient from experiments, and the permeability of Dex through the materials was the greatest. Thus, the results suggest that Dex diffusion through the polymer capsule is not mainly through the pores of the capsule membrane, but through the dissolution in the membrane.

In the modeling, because Kr and τ were not experimentally determined, the ratio of Kr/τ was fit to the actual release data. Many studies have made an effort to establish the relationship between tortuosity, τ, and porosity, ε [22,23]. ε is a function of void volume over total volume. The void volume can be estimated using the volume of single pore times the number of pores inside the members. By assuming the shape of the pores as spheres, the volume of a single pore is $4/3\ \pi r_p^3$, where r_p is the radius of the pore. Thus, porosity is proportional to r_p^3. On the other hand, Kr is proportional to $\left[1-\frac{d_m}{\varepsilon^{-1/3}}\right]^4$ by substituting the relationship between pore diameter and porosity into the equation. This derivation indicates that as porosity increases, the ratio of Kr/τ decreases. Porosity is inversely proportional to the ratio of Kr/τ. In our study, PCL, with the smallest porosity measured as 0.0577, has the largest Kr/τ ratio of 0.001169, 90 : 10 PLGA with the largest porosity measured as 0.0986 having the smallest Kr/τ ratio of 0.000721, and the other two polymers have very similar porosity and Kr/τ ratio, which follows the derivation.

In addition to physiochemical characterization of polymer implant, we also explored its cytotoxicity and biological application using in vitro cell tests. According to literature, HUVEC stimulated with TNF-α produces proinflammatory mediators, which will aggravate endothelial dysfunction [24-26]. Our experimental findings show that Dex or Dex-loaded implant reduces HUVECs inflammation induced by TNF-α by decreasing the expression of pro-inflammatory cytokines, IL-6. Most interestingly, the IL-6 concentration in the cell culture medium significantly decreased in the HUVECs treated with Dex-loaded implant than the free Dex, which is probably due to the sustained release from the implant over time that can persistently inhibit IL-6 expression. Hence, the dosing method is highly important for effective Dex delivery to inhibit the expression of pro-inflammatory cytokines, which in turn enhances its anti-inflammatory effects. Although statistically not significant (p=0.095), the IL-6 level from the negative control (condition 1) is lower than condition 5. This may be due to the lower cell viability level of condition 5 than condition 1, as shown in Fig. 7. Similarly, the reason why the IL-6 level from the PBS-implant (condition 3) is less than condition 2, although statistically not significant (p=0.078) is also probably because of the lower cell viability of condition 3 compared to condition 2. Interestingly, HUVEC treated with free Dex (condition 4) showed the highest cell viability and IL-6 level. We suspect this is because the cell was treated with Dex prior to the TNF-α administration. Some literature showed that Dex increases HUVEC proliferation in the presence of VEGF [27] and pro-inflammatory responses when Dex is administered prior to TNF-α [28].

Finally, the MTT viability assay showed that more than 80% of HUVECs were alive in all treatment groups, indicating that synthesized polymer implant or Dex at tested concentration has minimal toxicity to HUVECs.

CONCLUSION

We demonstrated the release trend of Dex inside the polymer tubes is dependent on the hydrophobicity of the drug and the polymer, in which PCL releases Dex in the form of DSP slowest. Furthermore, by testing the permeability and partition coefficient of each

polymer, the release kinetics of different polymers can be predicted using first-order kinetics modeling for a cylinder reservoir shape with known dimensions of the capsules. Finally, our cell test proved that the PCL Dex implant decreases IL-6 concentration more compared to the free Dex due to a sustained release over 48 h with minimum cell viability.

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SUPPORTING INFORMATION

Additional information as noted in the text. This information is available via the Internet at http://www.springer.com/chemistry/journal/11814.

REFERENCES

1. A. M. Campagnolo, D. H. Tsuji, L. U. Sennes, R. Imamura and P. H. N. Saldiva, *Ann. Otol. Rhinol. Laryngol.*, **119**, 133 (2010).
2. R. O. Graham and G. A. Peyman, *Arch. Ophthalmol.*, **92**, 149 (1974).
3. K. Heimdal, H. Sletteb, K. Watne and O. J. Nome, *Neurooncol.*, **12**, 141 (1992).
4. K. Kishore, P. Venkatesh and C. C. Canizela, *Clin. Ophthalmol.*, **16**, 1019 (2022).
5. J.-E. Chang-Lin, M. Attar, A. Acheampong, M. Robinson, S. Whitcup, B. D. Kuppermann and D. Welty, *Invest. Ophthalmol. Vis. Sci.*, **52**, 80 (2011).
6. R. Herrero-Vanrell, J. A. Cardillo and B. D. Kuppermann, *Clin. Ophthalmol.*, **5**, 139 (2011).
7. R. Bhagat, J. Zhang, S. Farooq and X. Y. Li, *J. Ocul. Pharmacol. Ther.*, **30**, 854 (2014).
8. M. Mohammadi, K. Patel, S. P. Alaie, R. B. Shmueli, C. G. Besirli, R. G. Larson and J. J. Green, *Acta. Biomater.*, **73**, 90 (2018).
9. O. Pillai and R. Panchagnula, *Curr. Opin. Chem. Biol.*, **5**, 447 (2001).
10. C. B. Chng, J. Q. Choo and C. K. Chui, *Acta. Biomater.*, **8**, 2835 (2012).
11. J. W. Choi and J. K. Park, *Macromol. Biosci.*, **17** (2017).
12. Y. H. Choi, H.-J. Ahn, M. R. Park, M.-J. Han, J. H. Lee and S. K. Kwon. *Acta. Biomaterialia.*, **86**, 269 (2019).
13. J. M. S. Coppoolse, T. G. V. Kooten, H. K. Heris and L. Mongeau, *J. Speech Lang. Hear. Res.*, **57**, S658 (2014).
14. E. Elibol, Y. F. Yilmaz, A. Unal and M. Ozcan, *Eur. Arch. Otorhinolaryngol.*, **278**, 1537 (2021).
15. I. Vroman and L. Tighzert, *Materials*, **2**, 307 (2009).
16. E. J. Freney, S. T. Martin and P. R. Buseck, *Aerosol Sci. Tech.*, **43**, 799 (2009).
17. H. Wen, J. Hao and S. K. Li, *J. Pharm. Sci.*, **102**, 892 (2012).
18. J. Siepmann and F. Siepmann. *J. Control. Release*, **161**, 351 (2011).
19. P. Grathwohl, *Diffusion in natural porous media*, Springer US: Boston, MA, 43-81 (1998).
20. T. Waterkotte, X. He, A. Wanasathop, S. K. Li and Y. C. Park, *ACS Biomater. Sci. Eng.*, **8**, 4428 (2022).
21. H.-X. Hao, J.-K. Wang and Y.-L. Wang, *J. Chem. Eng. Data*, **49**, 1697 (2004).
22. J. Siepmann and F. Siepmann, *J. Control. Release*, **161**, 351 (2012).
23. A. A. Garrouch, L. Ali and F. Qasem. *Mater. Interfaces*, **40**, 4363 (2001).
24. J. Fu, H. R. Thomas and C. Li. *Earth-Sci. Rev.*, **212**, 103439 (2021).
25. T. Bian, H. Li, Q. Zhou, C. Ni, Y. Zhang and F. Yan, *Mediators Inflamm*, **2017**, 8529542 (2017).
26. S. Wu, H. Xu, J. Peng, C. Wang, Y. Jin, K. Liu, H. Sun and J. Qin, *Biochimie*, **110**, 62 (2015).
27. Z. Jia, P. V. Babu, H. Si, P. Nallasamy, H. Zhu, W. Zhen, H. P. Misra, Y. Li and D. Liu, *Int. J. Cardiol.*, **168**, 2637 (2013).
28. E. Langendorf, P. M. Rommens, P. Drees and U. Ritz, *Int. J. Mol. Sci.*, **22**, 7986 (2021).
29. M. G. Frank, Z. D. Miguel, L. R. Watkins and S. F. Maier, *Brain Behav. Immun.*, **24**, 19 (2010).