Acute and chronic issues, such as scarring, can cause various complications that adversely impact or compromise the performance of implants, and even lead to a secondary surgery.5 These devices remarkably improved the life quality of millions of patients worldwide. The formation of a scarlike capsule isolating medical devices from adjacent tissues in vivo, but often results in excessive peri-implant capsule thickness, production and deposition of collagen, and expression of contracture-mediating factors compared with bare silicone implants. More importantly, such an optimum dose had an excellent repeatability for the suppression of the capsular formation. Therefore, this study provides a strategic foothold regarding the sustained release of low-dose PTX to alleviate fibrotic capsule formation after implantation, and the microgram-level PTX-loaded thermogel holds great potential as an “all-purpose antiﬁbrosis coating” for veiling the surfaces of various implantable medical devices.

KEYWORDS: thermogel, paclitaxel, ﬁbrous capsule, silicone implant, capsular contracture, sustained drug delivery
Paclitaxel (PTX) is a famous chemotherapy drug that disrupts the normal microtubule dynamics by stabilizing the microtubule and increasing microtubule polymerization, and eventually induces cell apoptosis.\textsuperscript{15,16} It has been extensively used in the treatment of various types of cancers. Interestingly, recent studies indicate that low-dose PTX has good inhibitory effects on the synthesis of collagen, growth and proliferation of fibroblasts, and neovascularization,\textsuperscript{16,17} and has been tried to treat collagen-induced arthritis, multiorgan fibrosis, and fibrosis-related systemic sclerosis.\textsuperscript{18–22} We thus hypothesized that low-dose PTX may be therapeutic for hypertrophic fibrosis scarring around implantable medical devices. No pertinent report has been found, and the present study aims to check our hypothesis in combination with the unique thermogelling materials.

Compared with systemic administration, localized drug delivery may significantly enhance therapeutic efficacy while minimizing systemic toxicity. In situ-forming injectable hydrogels are very attractive as localized drug carriers because of their superior advantages including facile fabrication, target administration, and a sustained drug release manner.\textsuperscript{23–28} Injectable thermogels are a type of hydrogels that are free-flowing polymer solutions at low or room temperature, but turn into semisolid hydrogels at body temperature.\textsuperscript{29–39} Therefore, drugs can be easily entrapped into the polymer solutions by simply mixing them at a low temperature, whereas drug-loaded thermogel depots can be spontaneously formed upon injection into the target sites. Among them, biodegradable and biocompatible thermogels based on poly(lactic acid-co-glycolic acid)-b-poly(ethylene glycol)-b-poly(lactic acid-co-glycolic acid) (PLGA-PEG-PLGA) triblock copolymers are one of the most popular systems owing to the good profile of biocompatibility and the long persistence in the gel form in vivo, and have been employed to deliver a variety of drugs to treat different diseases, such as cancer, osteopenia, and so forth.\textsuperscript{30,40–44}

In the present work, we suggest using the local, sustained release of low-dose PTX around silicone implants to mitigate capsular formation and/or contracture, as schematically illustrated in Figure 1. First, a thermogelling triblock copolymer, PLGA-PEG-PLGA, was synthesized and the sol–gel transition of the PLGA-PEG-PLGA aqueous solutions with or without PTX was studied. Subsequently, we examined the in vitro drug release behaviors of the thermogel formulations containing microgram-level PTX. Silicone implants coated with microgram-level PTX-loaded thermogels were inserted into the subpanniculus plane of rats, and the resulting fibrosis capsules were carefully compared with those obtained from bare silicone implants. Various quantitative analyses comparing capsular thickness, inflammatory cellularity, vascularity, and amounts of transforming growth factor-β (TGF-β), α-smooth muscle actin (α-SMA), and CD68 were performed to access the effects of the sustained release of PTX with varied drug doses on peri-implant capsular formation. The optimum drug dose window was determined and its repeatability was further validated. This study provides a new way to reduce the excessive fibrosis scarring after implantation, including capsular contracture through the sustained release of low-dose PTX at the local insertion site.

2. MATERIALS AND METHODS

2.1. Materials. PEG with a molecular weight (MW) of ~1500 and a molar mass distribution (Dₚ) of ~1.13, and stannous octoate (Sn(Oct)₂, 95%) were purchased from Sigma-Aldrich. Monomers D,L-lactide (LA) and glycolide (GA) were products of Jinan Daigang Biomaterial Co., Ltd. (China). PTX (99.8%) was obtained from Shanghai Meishidun Biotechnology Co., Ltd. (China). Miniature silicone breast implants identical to implants used in clinical breast surgery in terms of materials and texture (textured type) with an average weight of 1.38 ± 0.10 g, diameter of 15 mm, and height of 10 mm were customized from Shanghai Winner Plastic Surgery Products Co., Ltd. (China). Clinical Taxol solution [each milliliter of solution contains 6 mg of PTX, which is solubilized by Cremophor EL (polyoxyethylated castor oil)] was supplied by the Obstetrics and Gynecology Hospital of Fudan University and produced by Hainan Haiyao Co., Ltd. (China). All other reagents and materials were used as received.

2.2. Animals. Female Sprague-Dawley (SD) rats (average weight: 220–250 g) were purchased and housed at Shanghai Laboratory, Animal Research Center (China). The animals were raised in an environment of 20–24 °C, relative humidity of 30–70%, and 12 h light/dark cycle with free access to standard rat chow and water ad libitum. All animal experiments were performed under the approval of the ethics committee of Fudan University.

2.3. Polymer Synthesis and Characterization. The triblock copolymer PLGA-PEG-PLGA was synthesized by the ring-opening polymerization of LA and GA initiated by the hydroxy end group of PEG and catalyzed via Sn(Oct)₂.\textsuperscript{45} Briefly, 15 g PEG was first added into a three-neck flask and then dehydrated under vacuum at 120 °C for 3 h. Next, given amounts of LA, GA (molar ratio 3/1), and catalyst Sn(Oct)₂ were introduced into the reaction device at 90 °C under the protection of argon and then dehydrated for another 30 min. Afterwards, the temperature of the device was raised to 150 °C for 12 h with continuous stirring. After the completion of the reaction, the crude products were purified by washing in 80 °C hot water three times and then lyophilized to eliminate the residual water. Finally, the products were collected and stored at ~20 °C until use.

\textsuperscript{1}H NMR was applied for structure and MW estimation of the polymer on a 400 MHz NMR spectrometer (AVANCE III HD 400 MHz, Bruker) using CDCl₃ as the solvent and tetramethylsilane as the internal reference. The MW and Dₚ of the polymer were further determined by gel permeation chromatography (GPC, Agilent 1260) equipped with a refractive index detector. The measurement was performed at 35 °C using tetrahydrofuran as the eluting solvent at a flow rate of 1.0 mL/min, and monodispersed polystyrene was used as the calibration standard of MW. The sol–gel transitions of 25 wt % PLGA-PEG-PLGA triblock copolymers in normal saline (NS) with or without drugs were monitored using a dynamic stress-controlled rheometer (Malvern, Kinexus) equipped with a cone-plate (diameter: 60 mm, cone angle: 1°). Storage modulus G’ and loss modulus G”
were recorded with an oscillatory frequency \( \omega \) of 10 rad/s and a heating rate of 0.5 °C/min from 15 to 50 °C. The morphology of micelles was observed with a transmission electron microscope (TEM, Tecnai G2 20 TWINS, FEI) with an accelerating voltage of 200 kV. Samples were prepared by placing one drop of the polymer solution (1 wt %) on a copper grid coated with a superthin carbon film, dried under an infrared lamp, and observed without staining.

2.4. In Vitro PTX Release. Polymer aqueous solutions (25 wt % in NS) containing 80, 40, and 20 \( \mu \)g/mL PTX were prepared by directly mixing the polymer solution with the drug and stirring them at 4 °C. One milliliter of PTX-loaded PLGA-PEG-PLGA copolymer aqueous solution was injected into a 15 mL vial (outer diameter: 22 mm) and then incubated in a shaking bath (37 °C, 50 rpm) for 15 min to form a stable physical hydrogel containing PTX. Next, 10 mL of prewarmed phosphate buffer saline (PBS, pH 7.4) solution containing 0.025 wt % sodium azide and 0.5 wt % Tween-80 was added as the release medium. Half of the release medium was replaced with the same amount of fresh medium every 3 days to maintain the sink condition. At specific time points, after decanting the release media in vials (n = 3 for each time point), the remaining hydrogels containing PTX were collected and then lyophilized. The residual samples were re-dissolved in acetonitrile (2 mL per sample). After centrifugation, the supernatants were analyzed by a high-performance liquid chromatography (HPLC) system (Waters e2695) equipped with a SunFire C18 reverse-phase column (4.6 × 150 mm, 5 \( \mu \)m) and a UV-vis detector (Waters 2489). The mobile phase consisted of a mixture of water and acetonitrile in a 50:50 (v/v) ratio and was delivered in the isocratic elution mode at a flow rate of 1.0 mL/min. UV–vis detection at 227 nm was used for the quantification of PTX. The accumulated release amount was obtained based on a PTX standard calibration curve prepared with acetonitrile (R² > 0.9999) as shown in Figure S1.

The release profiles of the PTX-loaded hydrogel formulations (80, 40, and 20 \( \mu \)g/mL) were further fitted via a zero-order model \( M_t / M_\infty = kt \). Here, \( M_t \) means the cumulative release amount at the time of \( t \); \( M_\infty \) is the final release amount at an infinite time, and \( k \) is a constant.

2.5. In Vivo Degradation of Thermogel. In situ formation of the PLGA-PEG-PLGA thermogel and subsequent in vivo degradation were examined in an SD rat model. The animals were anesthetized by intraperitoneal administration of chloral hydrate (350 mg/kg), the backs of the rats were shaved, cleaned, and sterilized by irradiation of Co-60, was subcutaneously injected into the neck of the rats. The animals were sacri- ficed 0, 14, and 30 days from implantation. The mice were anesthetized by intraperitoneal injection of chloral hydrate. The scanning parameters were set as follows: spatial resolution of 18 \( \mu \)m pixel size; X-ray tube voltage of 80 kV; X-ray tube current of 300 \( \mu \)A; Al + Cu filter; 0.5° rotation step of 360°.

2.7. Micro-Computed Tomography. Micro-computed tomography (Micro-CT) (Bruker, Skyscan 1176) was applied to monitor the shape and contour of the implants after 0, 14, and 30 days from implantation. The mice were anesthetized by intraperitoneal injection of chloral hydrate. The scanning parameters were set as follows: spatial resolution of 18 \( \mu \)m pixel size; X-ray tube voltage of 80 kV; X-ray tube current of 300 \( \mu \)A; Al + Cu filter; 0.5° rotation step of 360°.

2.8. Histological and Immunohistochemical Analysis. The harvested specimens were embedded in paraffin and sectioned for hematoxylin and eosin (H&E) and Masson’s trichrome staining. Each stained slide was photographed under an inverted fluorescence microscope (Axiovert 200, Zeiss) equipped with a charge-coupled device (CCD) (AxioCam HRC, Zeiss) for later processing, and this process was performed under the circumstance of no access to the sample information. The capsule thickness around the implants was measured according to the previous publication with modifications. Briefly, the thicknesses of 10 sites, which were designated every 150 \( \mu \)m, were measured using the software Image J (NIH). The capsule thickness for each specimen was obtained by calculating the average of the thickness. Collagen deposition was observed and compared between groups from Masson’s trichrome staining.

Immunohistochemical staining was performed using anti-TGF-\( \beta \) (Abcam, UK), anti-\( \alpha \)-SMA (Abcam, UK), and anti-CD68 (Santa Cruz Biotechnology, USA). The stained sections were visualized using a Zeiss microscope (Axiovert 200) and imaged with a CCD camera at 200× magnification (AxioCam HRC, Zeiss) with the same imaging parameters. Again, all the images were obtained with the sample information masked from the operator. At each biopsy time and for each implant sample, three images were randomly obtained and analyzed.

The densities of the inflammatory cells and blood vasculature were quantified to measure the tissue response to the implant. Specifically, the number of inflammatory cells in the capsule of each image was calculated by Image-Pro Plus 6.0 (NIH) and data were presented as number per unit area (0.01 mm²). The number of blood vessels was counted manually for each image and expressed as a vessel number per unit area (1 mm²). For immunohistochemical staining analysis, the total pixel intensity of the formed capsule was measured using Image-Pro Plus 6.0 (NIH) and data were expressed as optical densities.

2.9. Statistical Analysis. Data analysis was performed with SPSS 16.0 (IBM Corp., USA). The results were expressed as means ± standard deviation (SD) or box-whisker plot. For data which were normally distributed with equal variances, one-way ANOVA with Tukey’s multiple comparison test was used. For the comparison between the two groups of the test–retest reliability, an unpaired \( t \)-test was used. A \( p \) value <0.05 was considered statistically significant.

3. RESULTS

3.1. Synthesis and Characterization of the PLGA-PEG-PLGA Triblock Copolymer. The PLGA-PEG-PLGA triblock copolymer was synthesized via the ring-opening copolymerization of LA and GA in the presence of PEG as the macroinitiator and Sn(Oct)\(_2\) as the catalyst, as presented in Scheme S1. The resultant specimen was characterized by \( ^1 \)H NMR and GPC measurements. All the characteristic proton
indicated that the drug release kinetics were controlled by the polymer/NS system and that no initial burst release was observed, but also that the cumulative release amount was more than 85%. Meanwhile, the release profile exhibited a sustained release manner up to 57 days. Not only did the injectability and thermogelling behavior of the PLGA-PEG-PLGA triblock copolymers improve, which is important for the maintenance of drug concentration in the remaining hydrogels, but also that the introduction of microgram-level PTX into the thermogel formulation was substantially successful, indicating the feasibility of the therapeutic use of PTX in the thermogel formulation containing 40 μg/mL PTX, as shown in Figure S5A.

Dynamic rheology analysis was further used to monitor the change of storage modulus \( G' \) and loss modulus \( G'' \) of polymer aqueous solutions with or without drugs as a function of temperature. As presented in Figure S5B, at low and room temperatures, the moduli were low and the polymer aqueous solution underwent a sol-gel transition as the temperature increased. Notably, the polymer aqueous solution turned into a semisolid gel at body temperature (37 °C), which is important for its in vivo biomedical application. The sol-gel transition was well maintained for the hydrogel formulation containing 40 μg/mL PTX, as shown in Figure S5A. Dynamic rheology analysis was further used to monitor the change of storage modulus \( G' \) and loss modulus \( G'' \) of polymer aqueous solutions with or without drugs as a function of temperature. As presented in Figure S5B, at low and room temperatures, the moduli were low and the polymer aqueous solution underwent a sol-gel transition as the temperature increased. Notably, the polymer aqueous solution turned into a semisolid gel at body temperature (37 °C), which is important for its in vivo biomedical application. The sol-gel transition was well maintained for the hydrogel formulation containing 40 μg/mL PTX, as shown in Figure S5A.

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the hydrogel was degraded via hydrolysis of PLGA blocks and the volume of the hydrogel gradually swelled, a clear interface between the release medium and the hydrogel was well maintained at this stage. Subsequently, tiny hydrogel fragments continuously broke away from the gel matrix and entered into the release medium because of the continuous degradation of the hydrogel, and thus the volume of the residual gel gradually decreased. At the end of the experiment (57 d), only a few portions of the remaining hydrogels were observed at the bottom of the vials.

3.4. In Vivo Degradation of the PLGA-PEG-PLGA Hydrogel. In situ gel formation and in vivo degradation of the PLGA-PEG-PLGA thermogel were confirmed and tracked in SD rats. The copolymer aqueous solutions rapidly transformed into in situ thermogels with round or irregular shape protrusion after subcutaneous injection into the neck of the animals. As displayed in Figure 4, the sizes of the hydrogels gradually decreased as a function of degradation time because of the hydrolysis of ester bonds of the PLGA segments. The final degradation products are lactic acid, glycolic acid, and nondegradable PEG, which are easily absorbed and metabolized in the body. The in vivo integrity of the gel was maintained for over 3 weeks and no visible residual hydrogel was detected at day 30 postinjection. Therefore, the in vivo persistence of the current PLGA-PEG-PLGA hydrogel was determined to be around 1 month.

Furthermore, no pathological symptoms such as tissue necrosis, hemorrhaging, hyperemia, edema, or muscle damage were observed during the whole in vivo experiment, suggesting the good biocompatibility of the PLGA-PEG-PLGA thermogel, which will be further supported by the subsequent histological results. It is worth pointing out that the in vivo maintenance of the PLGA-PEG-PLGA thermogel was shorter than that in vitro. This was attributed to the complicated physiological environment in the body, such as invasion of cells, enzymatic hydrolysis of ester bonds, and so on that accelerated the in vivo degradation of the PLGA-PEG-PLGA thermogel.

3.5. In Vivo Capsular Formation. To study the effect of the PTX-loaded hydrogel formulations on the formation of capsules around the implants, customized silicone implants whose surfaces were uniformly coated with a layer of hydrogel with or without PTX were inserted into the subpanniculus carnosus plane of the rats. All the subject animals survived during the entire examination period. The animals were sacrificed after 30 days from implantation. The tissues around the silicone implants were carefully harvested, embedded, and stained for further analysis.

3.5.1. In Vivo PTX Dosage-Dependence Study. To begin with, a preliminary in vivo PTX dosage-dependence study was carried out to determine the proper PTX loading amount in the thermogel formulations. Considering that the loading amount of PTX in the thermogel formulation (OncoGel) for the treatment of cancer generally reached the milligram level per milliliter, the PTX dosage was reduced by 1 order of magnitude (PTX 0.1, 0.2, and 0.4 mg/mL) in the current study.

As shown in Figure 5, the fibrous capsules at the material–tissue interface have been formed after the introduction of customized silicone implants for 1 month, regardless of the treatment of PTX-loaded thermogel formulations. However, severe inflammatory responses were observed in the fibrous capsule tissues after the treatment of the three thermogel formulations with varied drug dosages and the degree of inflammatory response increased with the increase of the PTX amount in the thermogel system. The thickness of the fibrous capsules of the rats receiving the treatment of 0.1 mg/mL PTX-loaded thermogel formulation was comparable with that of the NS group, yet the fibrous capsule contained a large amount of inflammatory cells (mostly lymphocytes) and fibroblasts. As the PTX concentration increased to 0.2 mg/mL, an intense inflammatory response in the capsule tissue resulted in a significantly increased thickness of the fibrous capsule. As for the animals treated with the thermogel...
formulation containing 0.4 mg/mL PTX, inflammatory cells even infiltrated into the adjacent muscle layer and the skin necrosis around the implantation sites was clearly observed during the experiment, indicating the presence of severe tissue toxicity. The preliminary PTX dosage-dependence study revealed that the PTX concentrations ranging from 0.1 to 0.4 mg/mL were still excessive, which caused obvious toxicity against the surrounding subcutaneous tissue. Therefore, the PTX loading amount was further reduced by another order of magnitude in the subsequent experiments.

3.5.2. In Vivo Microgram-Loaded PTX Study. The effects of the microgram-level PTX-loaded thermogel formulations on the formation of the fibrous capsule were further evaluated. Figure 6 shows the representative histological images of sections after the implantation of customized silicone implants combined with the different treatments for 1 month. In the case of the NS group, the capsules were rich in fibroblasts, histiocytes, and vascular endothelial cells. Meanwhile, the deposited collagen fibers in the capsule were dense and irregularly arranged. In contrast, the collagen fibers that formed in the blank thermogel (Gel) group were loose and showed a relatively parallel arrangement. The structure of the fibrous capsule in the PTX 20 group was similar to that of the Gel group. Interestingly, as the concentration of PTX increased to 40 μg/mL, the thickness of the fibrous capsule decreased notably and the parallelly aligned collagen fibers were highly loose. However, with further increasing the concentration of PTX to 80 μg/mL, both the significantly increased inflammatory cellularity and thickness of the fibrous capsule were found compared with that of the PTX 40 group.

3.6. Capsule Thickness. The differences in the fibrous capsules that formed in the different groups were further analyzed quantitatively. As shown in Figure 7A, the thickness of the fibrous capsule was measured every 150 μm and the average value of 10 sites was defined as the thickness of the tested capsule. In consistency with the morphological observations shown in Figure 6, the PTX 40 group exhibited the thinnest capsule thickness among the five groups (Figure 7B). Compared with the capsule formed in the PTX 40 group, the average capsule thickness was increased by 62% in the NS group, by 21% in the Gel group, by 33% in the PTX 20 group, and by 66% in the PTX 80 group. Meanwhile, significant statistic differences were observed between the PTX 40 group and the NS group or the PTX 80 group. This finding indicated that the administration of the 40 μg/mL PTX-loaded thermogel formulation and the subsequent sustained release of the drug remarkably alleviated the thickness of the formed capsule after the implantation of the silicone implants.

In order to further verify the repeatability or test–retest reliability, the effect of the most effective PTX concentration (40 μg/mL) versus the NS on the capsule thickness was studied again under the same experiment conditions. Figure 7C shows that the average capsule thickness in the PTX 40 group was 167 μm, whereas that in the NS group increased by 36% and reached 227 μm. Also, there was a significant statistic difference between the two groups. Therefore, the good repeatability of the efficacy of the optimized PTX-loaded thermogel formulation for the alleviation of capsule formation was validated.

Also, an independent control experiment was performed by the direct injection of the PTX solutions at the same low doses
(20, 40, and 80 μg/mL) around the silicone implants to evaluate whether the treatment effects were related to long-acting release or fast release of the drug. As presented in Figure S6, the short-acting administration of PTX solutions at the insertion site did not mitigate the capsule formation. Conversely, significant increases in capsule thickness (19, 33, and 53% higher than that of the control group on average for the Taxol 20, 40, and 80 groups, respectively) and inflammatory response were observed as the injection dose increased, indicating that the fast release of PTX around the silicone implants caused tissue toxicity and induced excessive fibrosis even if at the low doses. Obviously, this finding further confirmed that the alleviation of capsule formation in the PTX 40 group was attributed to the sustained release of proper dose of the drug at the insertion site.

3.7. Cellularity and Vascularity. Inflammatory cells act as a key indicator in inflammatory reactions, which secrete different cytokines, recruit fibroblasts, and activate collagen synthesis, and thus result in capsule formation. The density of intracapsular inflammatory cells (mostly lymphocytes with a small amount of monocytes and macrophages) was evaluated and is presented in Figure 8A. The PTX 40 group showed the narrowest data distribution with the smallest data of median (6.8 counts per 0.01 mm²) and average (7.6 counts per 0.01 mm²) among the testing groups. The declining trend of inflammatory cell counts was consistent with the capsule thickness results.

We also compared the vascularity, an indicator of neoangiogenesis, of the fibrous capsules around the implants (Figure S7). Compared with the other four groups, the PTX 40 group exhibited an overall trend of lower vascularity (Figure 8B). Meanwhile, we observed a significant difference between the PTX 40 group and the NS group.

3.8. In Vivo Micro-CT. To track the possible changes in the shape and contour of the implants caused by capsular contracture, the implants were scanned by micro-CT at designed intervals and then the three-dimensional morphologies of the implants were reconstructed. As shown in Figure S8,
there was no obvious change in both the shape and the contour of the implants and their transverse sections during the 1-month experiment. The reason for this negative result might be that the capsules formed around the implants within 1 month are not sufficient to cause a detectable compression by micro-CT. Though no change in the shape of the implants was observed, a noninvasive detection method was successfully applied by us to track the contour of the implants, which might have some reference for other researchers.

3.9. Immunohistochemical Analysis of Capsular Formation. We further performed immunohistochemical analysis to obtain more information on the capsular formation around the silicone implants. The level of TGF-β expression is a major cytokine secreted by inflammatory cells and fibroblasts. It has been regarded as a key mediator in the progression of fibrosis, which could promote the recruitment and proliferation of fibroblasts, activate fibroblasts to synthesize collagen, and downregulate matrix metalloproteinases.

One month after implantation, the optical density of TGF-β in the PTX 40 group was significantly lower than those in the NS, PTX 20, and PTX 80 groups (Figure 9A,D). We expected that the low level of TGF-β downregulated the inflammatory response and collagen synthesis, thus markedly alleviating the formation of the periprosthetic fibrous capsule.

α-SMA, as a sign of the formation of myofibroblasts, is responsible for the increased collagen production, extracellular matrix secretion, and fibrotic lesions. Although no significant difference was observed among the testing groups, the optical density of the PTX 40 group showed a lower data distribution than the NS, Gel, and PTX 80 groups (Figure 9B,E). The low titer of α-SMA in the PTX 40 group would contribute to the decrease of collagen deposition (as shown in Masson’s trichrome staining images) and the suppression of capsular formation.

CD68, a typical inflammatory cell marker, was also related with the severity of fibrosis. There was no significant difference among the testing groups. However, it is still worth pointing out that the PTX 40 group presented a relatively low CD68 expression compared with the other four groups (Figure 9C,F). This finding is consistent with the results of the inflammatory cell counts in Figure 8A.

4. DISCUSSION

Capsular contracture remains the most troublesome postoperative complication after the breast implant insertion. Although the underlying mechanism of capsular contracture is still controversial, excessive foreign body reactions have been acknowledged as one of the key factors to cause the capsular contracture. Typically, the foreign body reactions involve the inflammation process from the acute to chronic stages, collagenous accumulation, and eventual formation of fibrous tissue encapsulating the implant within 3 weeks postsurgery. Surface modifications have been tried to alleviate the excessive foreign body reactions around the silicone implants, however, only limited success has been achieved in clinical studies.
Recently, researchers have shown that low-dose PTX is able to alter microtubule dynamics without causing toxicity and thus offers a potentially appealing option to treat various diseases associated with excessive fibrosis and inflammation.59 For example, Lv et al. found that the pulmonary fibrosis in a bleomycin-treated rat model was effectively ameliorated by intravenous injection of low-dose PTX (0.6 mg/kg daily for 2 weeks) to suppress the TGF-β1/Smad3 pathway.60 Liu et al. reported that the intraperitoneal injection of low-dose PTX (0.3 mg/kg, twice a week) significantly suppressed the tubulointerstitial fibrosis in a rat model of unilateral ureteral obstruction by inhibition of the TGF-β/Smad activity.21 Therefore, we hypothesized that local and sustained release of low-dose PTX may be a new therapeutic strategy to mitigate the capsular formation and/or contracture around silicone implants. Meanwhile, compared with the systemic administration, such as intravenous injection and intraperitoneal injection, local drug delivery can enhance the therapeutic potency while minimizing the drug-associated toxicities. To the best of our knowledge, no such long-acting delivery systems of low-dose PTX have ever been reported.

In the current study, we developed an injectable hydrogel consisting of thermogelling and biodegradable PLGA-PEG-PLGA triblock copolymers to deliver low-dose PTX for a long term around the silicone implants. As presented in Figure S9, the amphiphilic carrier polymers easily self-assembled into corona–core micelles in water. The concentrated polymer aqueous solution exhibited a macroscopic sol–gel transition as the temperature increased (Figure S5), and such a thermogelation process was attributed to the formation of a percolated micelle network via micellar aggregation.29,60 Because of the solubilization effect of polymer micelles, the hydrophobic PTX was conveniently encapsulated into the polymer aqueous solution simply by physical stirring at a low temperature. Meanwhile, the introduction of PTX did not obviously affect the injectability and temperature-induced sol–gel transition of the PLGA-PEG-PLGA thermogel (Figure S5).

A previous study has revealed that the milligram-level PTX-loaded PLGA-PEG-PLGA thermogel (ReGel) exhibited a sustained release manner of drug for approximately 50 days.50 Such a feature was also verified by us, as demonstrated in Figure S10. Interestingly, as the drug-loading amount decreased to the microgram scale, the release of PTX from the thermogel matrix similarly lasted up to 57 days and almost showed a constant rate throughout the whole examined period (Figure 2). This finding indicated that the carrier polymer degradation-controlled mechanism governed the PTX release regardless of the drug-loading amounts.

After insertion of the silicone implants into the subpanniculus carniosus plane of the rats, the microgram-level PTX-loaded polymer solution was instilled using a conventional syringe around the implants. Because of the contact with body heat, the polymer solution rapidly transformed into a thermogel depot containing PTX to veil the irregular surface of the implant. Subsequently, low-dose PTX was released in a sustained manner along with the gradual degradation of the thermogel matrix.

As is well known, the first month after implant insertion constitutes the most sensitive period of foreign body responses.13,57 In this study, we carried out a histological analysis of the tissues around the implants 1 month after the operation. In general, the capsule thickness is positively associated with the occurrence of capsular contracture.30 Our results revealed that the thickness of the capsule and inflammation level were dramatically dependent on the dose of PTX. When the drug loading amount in the thermogel matrix was greater than 80 μg/mL, the sustained release of PTX resulted in a remarkable enhancement of inflammation response in the capsule tissues (Figure 5). Further increasing the drug loading amount to 200 μg/mL, an intense inflammation response even led to a significant increase in the capsule thickness. This feature suggested that excessive PTX did not mitigate capsule formation, but caused serious side effects. In fact, previous studies have demonstrated that the dose of PTX plays a key role in maintaining the balance between the activity of fibrosis inhibition and the toxicity against normal tissues, and high-dose PTX induces inflammation and fibrosis.59,61

Interestingly, coating 40 μg/mL PTX-loaded thermogels on the surface of silicone implants notably reduced the capsule thickness, collagen density, and inflammatory cellularity (Figures 6–8). Nevertheless, a relatively low or relatively high loading amount of PTX (20 or 80 μg/mL) compromised the therapeutic efficacy so that no significant difference was detected compared with bare implants (Figures 6–9). Although the therapeutic window of PTX was relatively narrow, the repeatability test affirmed that the efficacy of the optimized PTX-loaded thermogel formulation for the alleviation of capsule formation was reliable and reproducible (Figure 7C). The good repeatability of the current study provides a great potential for the possible clinical translation in the future.

We further explored the mechanism of the efficacy of PTX for the treatment of capsular formation using the immunohistochemical analysis. Capsular contracture is a result of excessive foreign body responses because of acute and chronic inflammation after silicone implantation. In general, acute inflammation lasts up to several days. At this stage, neutrophils are predominant at first and then replaced by monocytes migrating from the vasculature as the predominant cell type.52 Subsequently, the persistent presence of the silicone implant leads to the transition from acute to chronic inflammation and the monocytes rapidly differentiate into CD68-positive macrophages.62,63 The macrophages (histiocytes) are known to produce growth factors and fibrocyte-stimulating cytokines.57,64 During the chronic inflammation stage, TGF-β1, a key mediator in the regulation of fibrosis,52,66 is secreted by persistently activated macrophages, lymphocytes, and fibroblasts.52,53 The production of TGF-β not only promotes the recruitment and proliferation of fibroblasts, but also stimulates fibroblasts to secrete collagen.53–55 Also, TGF-β drives the differentiation of fibroblasts into α-SMA-expressing myofibroblasts.53–55 Eventually, a contractile force in the capsule tissue caused by α-SMA-presenting myofibroblasts as well as excessive accumulation of collagen results in the fibrotic capsular contracture.12 Therefore, it is important to suppress the expression and activity of TGF-β1, α-SMA, and CD68 to prevent or mitigate the formation of capsular contracture around the silicone implants. In the current study, the administration of optimal PTX-thermogel formulation (40 μg/mL) and the subsequent sustained release of a proper dose of PTX significantly inhibited the expression of TGF-β1, α-SMA, and CD68 (Figure 9) and thus effectively alleviated the periprosthetic fibrous capsule formation.

In addition, the correlation between vascularity and capsule formation is still unclear and controversial. Lee et al. reported that vascularity was irrelevant to the capsule formation.13

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However, some researchers claimed that tissues containing more blood vessels were helpful to form softer capsules and reduce the risk of capsular contracture.\(^6\) In the current study, our results (Figure 8B) coincided well with the conclusions of some animal and clinical studies that a less-vascularized structure contributed to a reduced foreign body response around the implants, leading to the formation of thinner fibrous capsules and a lower probability of capsular contracture in breast augmentation.\(^{57,64}\) In the future, more research is called for to reveal the relationship between neoangiogenesis and capsular formation in implants.

It is also worth pointing out that the capsular contracture normally proceeds over 1 year.\(^{69}\) Therefore, the measurements at 1 month postimplantation are relatively short. Nevertheless, the initial process of capsular formation in the present model provides a good prediction of the overall foreign body reaction process. Besides, because of the complexity of foreign body reaction after implantation, a combination delivery system realizing a sustained codelivery of PTX and other drugs may bring about more effective efficacy and lead to lower incidence of capsular contracture.

Finally, overcoming the foreign body response to implanted biomaterials, which consists of inflammatory events and the wound-healing process, and eventually leads to fibrosis, is a critical need for developing medical devices and implementing new medical advances.\(^5,5\) Fortunately, various host responses following implantation of biomaterials share similar mechanisms.\(^7\) Therefore, the current study provides inspiring clues for other biomedical materials to resolve the fibrosis-associated complications following the implantation. This microgram-level PTX-loaded thermogel appears to be tremendously promising as an “all-purpose anti-fibrosis coating” for implantable biomedical devices because of the easiness and convenience of coating various surfaces without affecting their performances.

5. CONCLUSIONS

We propose the local, long-acting delivery of low-dose PTX as a new strategy to alleviate the capsule formation and/or contracture around silicone implants. A biocompatible and biodegradable thermogel composed of PLGA-PEG-PLGA triblock copolymers was prepared by us and used to encapsulate and deliver low-dose PTX. The microgram-level PTX-loaded thermogel system exhibited an almost perfect zero-order release profile in vitro up to 57 days, and the drug release profiles were not affected by changing the drug-loading amounts (PTX 20, 40, and 80 μg/mL). In vivo-formed fibrotic capsules around the silicone implants coated with the PTX-loaded thermogel with an appropriate dose (40 μg/mL) were significantly thinner. Besides, the capsules exhibited more regular collagen arrangement, lower inflammatory cellularity, vascularity, and expression of TGF-β, α-SMA, and CD68 than the bare silicone implants. Also, this optimum dose window had an excellent repeatability for the suppression of capsular formation. Consequently, we conclude that the sustained release of low-dose PTX at the local insertion site is a promising way to mitigate the capsule formation and/or contracture around silicone implants. This microgram-level PTX-loaded thermogel has great potential as an “all-purpose anti-fibrosis coating” for veiling surfaces of various implantable medical devices without affecting their functionalities.

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We propose the local, long-acting delivery of low-dose PTX as a new strategy to alleviate the capsule formation and/or contracture around silicone implants. A biocompatible and biodegradable thermogel composed of PLGA-PEG-PLGA triblock copolymers was prepared by us and used to encapsulate and deliver low-dose PTX. The microgram-level PTX-loaded thermogel system exhibited an almost perfect zero-order release profile in vitro up to 57 days, and the drug release profiles were not affected by changing the drug-loading amounts (PTX 20, 40, and 80 μg/mL). In vivo-formed fibrotic capsules around the silicone implants coated with the PTX-loaded thermogel with an appropriate dose (40 μg/mL) were significantly thinner. Besides, the capsules exhibited more regular collagen arrangement, lower inflammatory cellularity, vascularity, and expression of TGF-β, α-SMA, and CD68 than the bare silicone implants. Also, this optimum dose window had an excellent repeatability for the suppression of capsular formation. Consequently, we conclude that the sustained release of low-dose PTX at the local insertion site is a promising way to mitigate the capsule formation and/or contracture around silicone implants. This microgram-level PTX-loaded thermogel has great potential as an “all-purpose anti-fibrosis coating” for veiling surfaces of various implantable medical devices without affecting their functionalities.


