Postoperative pain is a complex physiological response to disease and tissue injury. Moderate-to-severe pain typically occurs within 48 h after surgery. Amino amide local anesthetics are widely applied to manage postoperative pain, and they have high efficacy, a low risk for addiction and limited side effects. However, these anesthetics also have short half-lives, often necessitating continuous injection to obtain satisfactory pain relief. In the current work, we used a poly(lactic-co-glycolic acid) (PLGA)–polyethylene glycol (PEG)–PLGA (PLGA–PEG–PLGA) temperature-sensitive gel to deliver a local anesthetic, ropivacaine hydrochloride (RP), to prolong its analgesic effect. We investigated the influence of polymer and drug concentration on gelation temperature and the in vitro drug release rate from the temperature-sensitive gel. RP-loaded PLGA–PEG–PLGA solution is a liquid at room temperature and forms a gel at temperatures slightly lower than body temperature. With regard to the gel's drug release rate, 37.5, 51.3 and 72.6% of RP was released at 12, 24 and 48 h, respectively. This in vitro drug release profile conformed to the Higuchi equation. To assess pain control efficacy when using the gel, we evaluated the mechanical paw withdrawal reflex threshold, thermal pain threshold and incision cumulative pain scores in a rat incisional model. The results showed that the anti-pain effect of a single injection of RP-loaded gel at the incision site lasted for 48 h, which is significantly longer than the effect produced by injection of RP solution alone. The use of RP-loaded thermosensitive gels could provide a promising method for managing postoperative pain.

Key words ropivacaine hydrochloride; thermogel; long acting; in vitro release; postoperative pain

Postoperative pain is a complex physiological response to disease and tissue injury. Such pain can induce various pathophysiological changes, which can affect the functions of the respiratory, circulatory, digestive and endocrine systems. If not well controlled, postoperative pain can also lead to severe immune and metabolic disorders. The most severe pain often occurs immediately after the operation, with pain intensity decreasing with time. It has been reported that moderate-to-severe pain most often occurs in the 48 h after surgery. Opioids are the most common treatment for such pain; however, they have numerous side effects, including nausea, vomiting, respiratory depression, prolonged ileus, itching, tolerance, and development of hyperalgesia. Topical application of local anesthetics is being increasingly used to relieve postoperative pain to reduce opioid requirements and improve analgesic effects.

Ropivacaine hydrochloride (RP) is a new amino amide local anesthetic with lower cardio- and neuro-toxicity than bupivacaine, making it particularly suitable for cardiac surgery and postoperative analgesia. Additionally, RP possesses lower lipid solubility and causes more vasoconstriction than bupivacaine, allowing better retention in the local environment. However, due to its short half-life ($T_{1/2}$ 1.8 h), RP must be repeatedly injected or continuously infused via a catheter to maintain its postoperative analgesic effects. This results in poor patient compliance and increases treatment costs. Furthermore, catheter infusion requires constant and meticulous monitoring to avoid accidental removal and to prevent complications, such as infection and nerve injury. Therefore, it is important to develop long-acting local anesthetics to manage postoperative pain.

Several sustained-release systems have been developed to deliver RP, including liposomes, microspheres and in situ implants; however, they all have limitations. RP-loaded poly(lactic-co-glycolic acid) (PLGA) microspheres were shown to achieve sensory and motor blockades for 48 h after implantation into the sciatic nerve in a mouse model. However, less than 50% of the drug was released within the first 48 h, and 192 h passed before 82% of the drugs were released. A novel RP-loaded in situ implant delivery system significantly prolonged the analgesic effect by 48 h compared with injection with RP alone. However, similar to the drug release rate via the PLGA microspheres, the drug release rate from the implant system became very low after 4 d: less than 50% of the drug was released in 2 d, and 14 d passed before 70% of the drug was released. Additionally, the use of N-methyl-2-pyrrolidone (NMP) in this system resulted in acute toxicity, with oral and dermal LD50 values ranging from 3600 to 7700 mg/kg in rodents. Thus, the safety of this injectable formulation should be further evaluated. A chitosan thermogel has been employed to deliver RP for regional musculoskeletal anesthesia. However, this gel requires the alteration of RP into an insoluble ropivacaine base via the addition of ammonium hydroxide, and only approximately 48% of the drug is released after 7 d.

In this present study, we developed a RP-loaded sustained-
release system that can extend pain relief to 48 h after surgery by local injection at the incision site. Compared with previous systems, the system developed here has an improved drug release profile, involves a simple technical process and can avoid organic solvent in the preparation. Thermosensitive gels have attracted increasing attention as injectable sustained-release drug-delivery systems. A triblock copolymer composed of hydrophobic PLGA and hydrophilic polyethylene glycol (PEG) blocks (PLGA–PEG–PLGA) shows thermosensitive sol–gel transitions and can form in situ hydrogels without harmful organic solvents or any chemical reactions.\(^1\,^2\) Additionally, the preparation of this copolymer is simple, and it offers convenient administration as it exists as a free-flowing liquid at room temperature that can readily be injected; at body temperature, it forms a cross-linked gel. Furthermore, PLGA–PEG–PLGA thermosensitive gels have been successfully used to deliver both hydrophobic and hydrophilic drugs.\(^1\,^2\,^3\)

In the present study, an RP-loaded PLGA–PEG–PLGA thermosensitive gel was designed for the purpose of prolonging pain relief after surgery. The effects of polymer and drug concentration on gelation temperature and in vitro drug release profiles were investigated. Pharmacodynamics and biocompatibility were evaluated in an incisional pain rat model.

### Experimental

#### Materials

RP was obtained from Jinan Hongfangde Pharmaceutical Company (Jinan, Shandong, China). PLGA–PEG–PLGA composed of PEG1000 and lactide–glycolide at a 3 : 1 ratio with \( M_n = 4000–5000 \) was obtained from Jinan Daigang Biotechnology Company (Jinan, Shandong, China). Sodium dihydrogen phosphate was purchased from Shanghai Experimental Reagent Company. Disodium hydrogen phosphate and sodium chloride were purchased from Sinopharm Chemical Reagent Company. Erythromycin ointment was obtained from Mayinglong Pharmaceutical Group Company. Acetonitrile and methanol were acquired from Tianjin Kermel Chemical Reagent Company.

#### Methods

**Preparation of PLGA–PEG–PLGA Thermosensitive Gels**

PLGA–PEG–PLGA polymer was added to phosphate-buffered saline (PBS, pH 8) and stirred until it was uniform liquid without visible clumps. The polymer continued to expand sufficiently at 4°C and became translucent. RP was slowly added to the PLGA–PEG–PLGA polymer solution and stirred continuously until the solution was homogeneous. The solution was then filtered through a Micro pore filter with a 0.2-μm pore-size membrane and maintained at 4°C until further use. Blank and RP PLGA–PEG–PLGA gel solutions were prepared at 25, 20, 15, and 10 and 5% (w/w) concentrations.

**Determination of Phase Transition Temperature**

Gelation temperature was determined using a tube inversion method.\(^7\) To accomplish this, a glass tube containing 1 mL PLGA–PEG–PLGA polymer solution was placed in a water bath. The temperature of the bath was then increased from 20 to 80°C at a rate of 1°C/min. At each 1°C interval, the tube was inverted to evaluate flowability. The solution was considered to have reached a gel state if no flow was observed for 30 s. The temperature at which the gel formed was then recorded as the gelation temperature. As the temperature increased, the gel precipitated. The temperature at which the gel precipitated was recorded as the precipitation temperature.

Each sample was measured three times.

**Rheological Characterization**

The viscoelastic properties of 20% (w/w) PLGA–PEG–PLGA gels containing 15 mg/mL RP were investigated using a HAKKE RheoStress 6000 Rheometer (Thermo Scientific, Newington, Germany). Temperature sweep tests were performed at a heating rate of 1°C/min within the range of ca. 20–40°C. The shear frequency was 1.0 Hz. A frequency sweep test was conducted at 25 and 37°C within the range of ca. 0.1–10 Hz.

**In Vitro Drug Release from PLGA–PEG–PLGA Thermosensitive Gels**

A 1-mL aliquot of the RP-loaded PLGA–PEG–PLGA polymer solution was added to a 10-mL test tube and maintained at 37°C in an air bath for 10 min. Subsequently, 5 mL of 0.9% sodium chloride was added, and the tube was maintained in a thermostatic air bath shaker at 37°C with a shaking rate of 50 rpm. The tube was sealed throughout the entire release process to prevent water evaporation. At predetermined time intervals, 4 mL of the release solution was withdrawn and replenished with an equal volume of fresh 0.9% sodium chloride. The samples were diluted and evaluated using HPLC on a Phenomenex C18 column (250×4.6 mm, 5 μm). The mobile phase consisted of phosphate buffer (0.01 mol/L, pH 4.0) and acetonitrile (75:25), and the UV detection wavelength was 263 nm. The flow rate was 1.0 mL/min, and the column temperature was 30°C. The in vitro release percentage at each time point was calculated as the ratio of the cumulative release amount to the total amount of RP added to the gel. Three replicates were performed for each formulation.

**Animals**

Sprague-Dawley (SD) rats (200–250 g) were purchased from Hubei Provincial Center for Disease Control (Wuhan, China) and housed in temperature and humidity-controlled systems. The study was approved by the Animal Care and Use Committee of Wuhan General Hospital of Guangzhou Command. The rats were divided into three groups: the first and second groups served as controls and received 0.2 mL 0.9% (w/v) sodium chloride and 1.5% (w/v) RP solution at 15 mg/kg, respectively. The experimental group was subcutaneously injected with a thermosensitive gel containing 1.5% (w/v) RP at 15 mg/kg.

**Incision Pain Model**

Cumulative pain score, heat pain threshold and mechanical paw withdrawal reflex threshold were measured in the rats before surgery to serve as a baseline. The rats were anesthetized using isoflurane. A 1-cm longitudinal incision was made under aseptic conditions through the skin and fascia, starting at 0.5 cm from the proximal edge of the heel and extending toward the toes, according to Brennan’s method.\(^8\) After using gentle pressure to achieve hemostasis, the skin was stitched with 2 needles, and 0.2 mL of the relevant solution was subcutaneously injected at the incision site. The entire surgical procedure took approximately 5 min and was always completed by the same person. Erythromycin ointment was used to provide protection from infection. The rats were housed in a quiet and warm environment.

**Determination of Mechanical Paw Withdrawal Reflex Threshold**

The rats were placed in a transparent acrylic glass box with a 1×1 cm aperture at the bottom and allowed to acclimate for
15 min. Each rat’s left hind foot was stimulated adjacent to the wound area using a BME-404 electronic mechanical measurement instrument (Chinese Academy of Medical Sciences Institute of Medical Biology, Beijing, China). When the rats exhibited quick flinching and licking, the stimulus was stopped and the pressure value (g) was recorded. The mechanical pain threshold was measured every 5 min, with 5 replicates, before surgery and again at 2, 4, 6, 12, 24, 48, and 72 h after surgery.

Determination of Thermal Pain Threshold

The rats were placed into a large beaker in a (55±0.5)°C water bath. When the left rear foot started tiptoeing, retracting, being licked, or showing hind limb retraction, the time was recorded as the thermal pain threshold. Each test was performed within 40 s with an approximate 10-min interval. The thermal pain threshold was measured in triplicate before surgery and at predetermined time points after surgery.

Determination of Incision Cumulative Pain Scores

Cumulative pain score was assessed by observing and comparing the position of each rat’s incised hind foot. If the hind foot blanched when pressed, it was considered to be in a weight-bearing position. When a rat’s hind foot was in a touchdown and weight-bearing position, 0 points were given. When the rat’s hind foot touched the floor without blanching, 1 point was given. When the rat’s hind foot was completely off of the floor, 2 points were given. Each animal was observed over a 1-min period every other 5 min for an hour. The final cumulative pain score was the sum of each score (0–24 points) measured during the scoring period. All processes were conducted in a mild and quiet environment to avoid a stress response. Each animal was assessed for cumulative pain score before surgery and at experimental time points after surgery.

Tissue Collection and Histology

The rats were sacrificed 7 d after drug administration. Each incision site was exposed and evaluated for signs of gross pathology. Muscle tissue at the incision site was collected, fixed in 10% paraformaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). Histological evaluations were performed by a pathologist from Wuhan General Hospital of Guangzhou Command (Wuhan, China).

Data Analysis

Data were statistically analyzed using ANOVA and Student’s t-test and are presented as the mean±standard deviation (S.D.). The level of significance was set at p<0.05.

Results and Discussion

Effect of Polymer Concentration on Phase Transition of a Blank PLGA–PEG–PLGA Solution

The PLGA–PEG–PLGA solution exhibited 4 physical forms as the temperature increased: a free-flowing solution (Fig. 1A), a transparent gel (Fig. 1B), an opaque gel (Fig. 1C), and a precipitate (Fig. 1D). The bridging connections among micelles facilitated the sol–gel transition but hindered the gel-precipitation transition. With the increase in polymer concentration, the bridging connections among micelles were enhanced. Thus, as shown in Fig. 2, the gelation temperature of the blank PLGA–PEG–PLGA solution decreased slightly as the polymer concentration increased while the precipitation temperature increased. These results are concordant with previous reports.7,20–22

Effects of Drug and Polymer Concentration on Gelation Temperature of an RP-Loaded PLGA–PEG–PLGA Solution

The gelation temperature of the RP-loaded 20% w/w PLGA–PEG–PLGA solution increased as the RP concentration increased (Table 1). When the RP concentration was 15 mg/mL, the gelation temperature was 35.8°C, which strengthened. The PLGA blocks can easily transit among located into different micelles, leading to bridging connections among individual micelles.20 Such connectivity reduced the mobility of the solution and resulted in a sol–gel phase transition.

As the temperature continues to rise, the hydrophobic interactions among the micelles are strengthened, and the aggregation of micelles increases to form a denser gel. At this stage, the transparent gel became opaque (Figs. 1B, C). As the temperature increased further, the hydrophobic PLGA blocks contracted tightly and the hydrophilic PEG blocks underwent dehydration, which resulted in the destruction of the micelle structures and the formation of a cloudy precipitate (Fig. 1D). The bridging connections among micelles facilitated the sol–gel transition but hindered the gel-precipitation transition. With the increase in polymer concentration, the bridging connections among micelles were enhanced. Thus, as shown in Fig. 2, the gelation temperature of the blank PLGA–PEG–PLGA solution decreased slightly as the polymer concentration increased while the precipitation temperature increased. These results are concordant with previous reports.7,20–22

Fig. 1. Four Physical Forms of PLGA–PEG–PLGA Solution

(A) Free-flowing solution. (B) Transparent gel. (C) Opaque gel. (D) Precipitation.

Fig. 2. Effect of Polymer Concentration on Phase Transition of PLGA–PEG–PLGA Solution

Table 1. Effects of RP Concentration on Gelation Temperature of a 20% (w/w) PLGA–PEG–PLGA Solution (n=3)

<table>
<thead>
<tr>
<th>PLGA–PEG–PLGA % (w/w)</th>
<th>RP (mg/mL)</th>
<th>Gelation temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>15</td>
<td>35.8±0.3</td>
</tr>
<tr>
<td>30</td>
<td>30</td>
<td>38.9±0.4</td>
</tr>
<tr>
<td>45</td>
<td>45</td>
<td>40.5±0.5</td>
</tr>
</tbody>
</table>
is slightly lower than body temperature. When the concentration of RP was increased from 30 to 45 mg/mL, the gelation temperature exceeded 37°C, which rendered the solution incapable of forming a gel at normal body temperature. Notably, we observed that the gelation temperature increased at higher RP concentrations, which is a different result from those found in previous reports of drug-loaded PLGA–PEG–PLGA gels.\(^\text{21,23–25}\) This discrepancy might be related to the interaction between the drug and the polymer. Some researchers have assumed that the hydrophilic or soluble salts of drug molecules, after absorbing water molecules themselves, can reduce the water activity and access of PEG blocks to the water molecules, thereby facilitating the phase transition at lower temperatures.\(^\text{20}\) However, such a mechanism cannot explain our results because RP is also a soluble drug. We assumed that the influence of drug concentration on the gelation temperature might be related with the concentration range of the drug. The interaction between RP and the hydrophilic polymer segment PEG might hinder the aggregation of micelles at high drug concentrations. Such a process could explain why the gelation temperature increased at high concentrations of RP.

The gelation temperature decreased as the polymer concentration increased (Table 2). When the PLGA–PEG–PLGA concentration was in the range of 15–25% and the RP concentration was 15 mg/mL, the solution was able to form gels at temperatures below body temperature. Thus, in vitro release studies were performed using these three formulations.

**Rheological Properties of RP-Loaded PLGA–PEG–PLGA Solutions** Thermosensitive gels have the rheological properties of both a sticky liquid and an elastic solid, which can be described using the viscosity modulus \(G'\) (loss modulus) and the elastic modulus \(G''\) (storage modulus), respectively. Figure 3 indicates the rheological properties of the 20% (w/w) PLGA–PEG–PLGA solution containing 15 mg/mL RP. At lower temperatures, \(G''\) was higher than \(G'\), and the system existed as a free-flowing liquid. As the temperature increased, \(G'\) and \(G''\) increased sharply, with \(G'\) increasing at a faster rate than \(G''\). As the value of \(G''\) became greater than that of \(G'\), the system entered a gel state and lost flowability. The sol–gel transition temperature was approximately 34.7°C, which corresponded to the point at which the \(G'\) and \(G''\) curves intersected. The sol–gel transition temperature was close to the result obtained using the tube inversion method (35.8°C). Below room temperature (approximately 25°C), the complex viscosity \(\eta^*\) was low with little correlation with temperature. During the onset of gelation, \(\eta^*\) increased sharply as the temperature increased. When the temperature reached the gelation temperature (approximately 34°C), \(\eta^*\) did not increase with further increasing temperature (Fig. 3B). At 25°C, the phase angle was constant at approximately 70° over the entire shear frequency range, indicating that the system was in a liquid state at room temperature. When the temperature increased to 37°C, the phase angle was reduced to approximately 23°, and the system formed a compact network, which facilitated a controlled drug-release rate (Fig. 3C).

**Effects of Drug and Polymer Concentration on in Vitro Release Profiles of 25% (w/w) PLGA–PEG–PLGA Thermosensitive Gels** As shown in Fig. 4, the drug release rate from the 25% (w/w) PLGA–PEG–PLGA thermosensitive gel was slightly reduced as the RP concentration increased from 15 to 30 mg/mL. For both formulations, the cumulative release percentage at 48 h was less than 60%, which was insufficient to effectively mediate postoperative pain relief.

Polymer concentration plays a more important role than drug concentration in RP release from thermosensitive gels. When the PLGA–PEG–PLGA concentration was increased from 15 to 25% (w/w), the drug release rate at 24 h was reduced from 81.0 to 33.3% (Fig. 5). This reduction might be

<table>
<thead>
<tr>
<th>RP (mg/mL)</th>
<th>PLGA–PEG–PLGA % (w/w)</th>
<th>Gelation temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>15</td>
<td>36.7 ± 0.1</td>
</tr>
<tr>
<td>15</td>
<td>20</td>
<td>35.8 ± 0.3</td>
</tr>
<tr>
<td>15</td>
<td>25</td>
<td>34.3 ± 0.3</td>
</tr>
</tbody>
</table>

Fig. 3. Viscoelastic Profile of 20% (w/w) PLGA–PEG–PLGA Solution Containing 15 mg/mL RP
(A) Elastic modulus \(G'\) and viscosity modulus \(G''\) curves. (B) Complex viscosity \(\eta^*\) as a function of temperature. (C) Phase angle as a function of frequency at 25 and 37°C.

Fig. 4. Effects of Drug Concentration on in Vitro Release Profiles of 25% (w/w) PLGA–PEG–PLGA Thermosensitive Gels
\(n=3\)
attributed to the increased polymer concentration: Due to the increase cross-links among the polymer molecules and subsequent increased viscosity of the hydrogel, the time required for the drug to diffuse from the gel might have been increased. This result is consistent with many previous studies.\textsuperscript{23,25} The in vitro release profile was consistent with the Higuchi equation ($Q = 0.1026t^{1/2} + 0.0141$, $r^2 = 0.9994$). RP was primarily released from the PLGA–PEG–PLGA gel through diffusion. This is concordant with previous studies showing that hydrophilic drugs mainly undergo diffusion-controlled release.

Moderate-to-severe pain most likely appears within 48 h after surgery. According to this timeframe, the release rate from the 15\% PLGA–PEG–PLGA gel was overly rapid, with 81.0\% of RP being released in 24 h, whereas the release rate from the 25\% PLGA–PEG–PLGA gel was overly slow, with only 62.3\% of RP being released in 72 h. For the 20\% PLGA–PEG–PLGA gel, the RP-release rates were 37.5, 51.3 and 72.6\% at 12, 24 and 48 h, respectively. The gelation temperature of this formulation was 35.8°C, which is suitable for injection. Thus, the 20\% PLGA–PEG–PLGA thermosensitive gel containing 15 mg/mL RP was chosen for pharmacodynamic evaluation.

Efficacy of Incisional Pain Relief A rat incisional pain model was first used in 1996 by Brennan to evaluate the effects of postoperative pain relief efforts.\textsuperscript{19} Incision pain relief efficacy was evaluated using three experimental parameters: mechanical paw withdrawal reflex threshold, thermal pain threshold and cumulative pain score. Figure 6 shows the incision pain relief efficacy results at each time point for the rats treated with the different regimens. Compared with the preoperative values, the postoperative mechanical paw withdrawal and the thermal pain thresholds were significantly reduced ($p<0.01$) for all groups at 2 h after surgery. The effects of the RP solution were comparable to those produced by the RP-loaded thermosensitive gel for a short period of time, as the mechanical paw withdrawal reflex and the thermal pain thresholds were significantly higher in the two RP groups compared with the blank control group at 2 h after surgery ($p<0.01$). However, the effects of the solution had diminished by 4 h. The mechanical paw withdrawal reflex and the thermal pain thresholds for the RP-loaded gel group were maintained at significantly higher levels than those for the RP solution and blank control groups ($p<0.01$ or $p<0.05$) for 48 h after surgery (Figs. 6A, B).
The cumulative pain score had significantly increased \( (p<0.01) \) 2h after surgery. The RP solution significantly lowered the cumulative pain score \( (p<0.01) \) for a short time after surgery, whereas the RP-loaded thermosensitive gel maintained a significantly lower cumulative pain score than the other two groups for at least 48h after surgery \( (p<0.01, \text{Fig. 6C}) \).

Overall, the mechanical paw withdrawal reflex threshold, thermal pain threshold and incision cumulative pain scores did not significantly differ between the RP solution and the RP-loaded thermosensitive gel groups 2h after surgery. However, the RP-loaded gel provided significant pain relief from 4 to 48h after surgery, whereas the RP solution did not (Fig. 6). Due to its instantaneous gelation upon injection, the RP-loaded gel showed significantly less diffusion from the administration site than the solution. As the RP underwent controlled release from the gel, a steadier and higher drug concentration could be maintained at the incision site compared with the RP solution.

**Local Tissue Responses to the RP-Loaded PLGA–PEG–PLGA Thermosensitive Gel** Muscle tissues at the incision sites were histologically evaluated 7d after injection. Overall, the organizational structure of the tissue was intact, and no gross tissue abnormalities were observed in any of the animals regardless of treatment. No significant differences were observed in pathological features among the blank, RP solution and RP-loaded thermosensitive gel groups (Fig. 7). These results demonstrate that the RP-loaded PLGA–PEG–PLGA thermosensitive gel has good biocompatibility. This biocompatibility likely resulted because the PLGA–PEG–PLGA triblock copolymer is readily biodegradable and can be hydrolyzed into water and carbon dioxide.

**Conclusion**

In this study, a long-acting locally injectable PLGA–PEG–PLGA thermosensitive gel was developed to deliver RP for postoperative pain control. Without the use of any organic solvents, the procedure of this sustained-release drug delivery system was simple and environmentally friendly. The gelation temperature and drug release rate could be adjusted by altering the polymer and drug concentrations. We found that a 20% PLGA–PEG–PLGA solution containing 15mg/mL RP formed a gel at temperatures close to body temperature and provided desirable in vitro release profiles. A single local injection of this formulation at the incision site of SD rats extended pain relief to 48h after surgery. No evidence of active inflammation or tissue irritation was observed, indicating that this long-acting in situ gel has good biocompatibility. We conclude that PLGA–PEG–PLGA thermosensitive gels provide a new modality for postoperative pain control.

**Acknowledgment** This work was supported by the Natural Science Foundation of Hubei Province (2013CFB430).

**Conflict of Interest** The authors declare no conflict of interest.

**References**


**Fig. 7.** Longitudinal Pathological Sections of Muscles 7d after Injection with (A) 0.9% NaCl, (B) RP Solution, or (C) RP-Loaded Thermosensitive Gel (×400)


