5-Fluorouracil loaded thermosensitive PLGA–PEG–PLGA hydrogels for the prevention of postoperative tendon adhesion

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Prevention of adhesion after tendon repair surgery is of considerable importance during tendon healing; however, current clinical outcomes are still not fully satisfactory. In this study, thermosensitive 5-fluorouracil (5-Fu) loaded PLGA–PEG–PLGA hydrogels were used as injectable physical barriers for the prevention of tissue adhesion during tendon healing and inhibition of fibroblast proliferation. The 5-Fu-loaded hydrogels showed a sol–gel–precipitation phase transition with increasing temperature, and the hydrogels displayed the maximum storage moduli at around physiological temperature. The sustained release of 5-Fu from the hydrogels lasted over 7 days. The PLGA–PEG–PLGA hydrogels degraded within 4 weeks after subcutaneous injection into rats, and showed acceptable biocompatibility in vivo. The anti-adhesion efficacy of the hydrogels, with or without 5-Fu, during the Achilles tendon healing of rats was evaluated by macroscopic and histological analysis. It was found that the group treated with 5-Fu-loaded hydrogels showed a significant inhibition of adhesion formation when compared to the untreated group or the group treated with the hydrogels only. Therefore, the 5-Fu-loaded injectable hydrogels hold potential as efficient physical barriers for the prevention of adhesion formation during Achilles tendon healing.

Introduction

The Achilles tendon is the most massive tendon in the human body and is mainly responsible for the function of ankle plantar flexion. The rupture incidence of the Achilles tendon is about 9.3 (±4.6)/100 000. The rupture of the Achilles tendon will lead to lower limb dysfunction, which will seriously affect the patients’ work and life. Therefore, an Achilles tendon rupture usually needs aggressive clinical treatments. Compared to conservative treatments, surgery can reduce the reoccurrence of rupture. However, tendon adhesion formed after surgery often results in complications such as pain, limited ankle movement, and even the requirement for secondary operation. Thus, how to prevent postoperative adhesion is one of the key problems for improving the clinical treatment efficacy.

The procedure of tendon healing includes extrinsic and intrinsic healing processes. Extrinsic healing is completed by the activation and differentiation of the subcutaneous and peritendinous fibroblasts, while the intrinsic healing is characterized by the activation, proliferation and migration of the endotenon cells. Extrinsic healing plays a key role in the occurrence of tendon adhesion, which usually induces the invasion of peripheral fibrous tissue into the tendon. It was found that postoperative adhesion can be effectively prevented by inhibition of extrinsic healing. Currently, various polymeric biomaterials have been applied as physical barriers around the tendon following tendon repair surgery to reduce adhesion formation, such as DegraPol polymer tube, collagen membrane, shear-aggregated fibronectin, and 2-methacryloyloxyethyl phosphorylcholine (MPC) hydrogels. However, perfect clinical outcomes using these materials are still limited. Generally, ideal anti-adhesion biomaterials should have merits such as favorable biocompatibility, biodegradability, ease of use and no adverse effects on tissue healing.

Hydrogels are three dimensional networks of hydrophilic polymers that can retain a large amount of water and display a semi-solid morphology. Injectable hydrogels, which may be formed in situ after injection of the polymeric precursor solutions into the body, have attracted considerable attention for biomedical applications as a result of their unique advantages including a simple and mild gelation process, minimally
invasive implantation procedure, good biocompatibility and biodegradability. Recently, several types of injectable hydrogels have been developed as physical barriers for the prevention of postoperative peritoneal adhesions. A significant reduction in the formation of peritoneal adhesion was observed after applying the hydrogels in the animal models of sidewall defect-cecum abrasion. Moreover, a MPC-based hydrogel was applied to reduce the peritendinous adhesion.

In addition to the physical barriers, various drugs, such as ibuprofen, indomethacin, corticosteroid, mannose-6-phosphate and mitomycin-C, have been used as anti-adhesion agents. As a kind of anti-metabolism and antitumor drug, 5-fluorouracil (5-Fu) can inhibit the proliferation of synovial fibroblasts and reduce the inflammation of surrounding tissue after tendon surgery. It was reported that 5-Fu has been used to prevent postsurgical tendon adhesions. It is noteworthy that the sustained release of 5-Fu in local tissue is crucial for achieving prolonged anti-adhesion efficacy. Recently, to achieve a sustained drug release of 5-Fu, injectable hydrogels were designed as depot release systems for localized, sustained tumor inhibition. Nevertheless, reports on the application of drug-loaded injectable and biodegradable hydrogels for preventing tendon adhesion are still limited. The applications of drug-loaded hydrogels as drug-releasing barrier materials can result in not only the formation of physical barriers for preventing cell adhesion, but also the construction of depot systems for localized and sustained drug release to attain an extended period of drug release.

In this work, 5-Fu-loaded injectable thermosensitive hydrogels based on a poly[D,L-lactide-co-glycolide]-poly(ethylene glycol)-poly[D,L-lactide-co-glycolide] (PLGA-PEG-PLGA) triblock copolymer were investigated for the prevention of postoperative Achilles tendon adhesion. The sol-gel phase transition of the 5-Fu-loaded hydrogels with increasing temperature was studied by a tube inverting method and rheology analysis. The in vivo degradation and biocompatibility of the hydrogels following subcutaneous injection into rats were evaluated. To reveal the efficacy of preventing postoperative tendon adhesion of the 5-Fu-loaded hydrogels in vivo, the hydrogels were applied to the Achilles tendon rupture model of rats, of which the Achilles tendons were cut off and sutured surgically. The anti-adhesion efficiency was analyzed by macroscopic evaluation and histological analysis.

Experimental

Materials
Poly(ethylene glycol) (PEG, $M_n = 1500$) and stannous octoate (Sn(Oct)$_2$, 95%) were obtained from Sigma-Aldrich (USA). Poly(D,L-lactide (LA) and glycolide (GA) were purchased from Purac (Netherlands). 5-Fluorouracil (5-Fu) was supplied by Vetec. 3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma-Aldrich (USA). L929 cells were obtained from the American Type Culture Collection (ATCC).

Synthesis of the PLGA-PEG-PLGA copolymer
The PLGA-PEG-PLGA triblock copolymer was prepared via ring-opening copolymerization of LA and GA by using PEG ($M_n = 1500$) as a macromolecular initiator. PEG was added into a three-necked flask and stirred under vacuum at 120 °C for 3 h to remove the trace water. After the flask was cooled to room temperature, LA and GA were added with a LA/GA molar ratio of 12 : 1, and Sn(Oct)$_2$ was added as a catalyst. The reaction was then allowed to proceed under nitrogen atmosphere at 130 °C for 24 h. Finally, the crude product was dissolved in cool distilled water and purified by heating to 80 °C to precipitate the copolymer and this process was repeated for 3 times. The final copolymer was obtained by lyophilization.

$^1$H NMR (300 MHz, CDCl$_3$, $\delta$ ppm): $\delta$ 1.5–1.6 (3H, –O–CH(CH$_3$)$_2$–CO–), $\delta$ 3.6–3.7 (4H, –(CH$_2$CH$_2$O)$_2$–), $\delta$ 4.2–4.4 (2H, –CO–CH$_2$CH$_2$O–), $\delta$ 4.6–4.9 (2H, –O–CH$_2$–CO–), $\delta$ 5.1–5.2 (1H, –O–CH(CH$_3$)$_2$–CO–). The molecular weight (MW) and polydispersity (PDI) of the resulting copolymer, determined by gel permeation chromatography (GPC), were 9680 and 1.18, respectively.

General characterization
$^1$H NMR spectrum of the triblock copolymer was obtained on a 300 MHz Bruker spectrometer (DMX300) at room temperature with CDCl$_3$ as the solvent. The chemical structure and composition were calculated by $^1$H NMR. Molecular weights and distributions of copolymers were obtained by gel permeation chromatography (GPC) at 35 °C. Chloroform (CHCl$_3$) was used as the eluent at a flow rate of 1.0 mL min$^{-1}$. Monodisperse polystyrene was used as a standard to generate the calibration curve for GPC.

Sol-gel phase transition
The PLGA-PEG-PLGA solutions in PBS (pH 7.4) with different weight concentrations (15, 20, and 25 wt%) were prepared at 4 °C. The sol–gel phase transitions of the triblock copolymer aqueous solutions were determined by using the vial inverting method as the temperature increased from 10 to 80 °C with a temperature interval of 1 °C per step. The sol-to-gel transition was recorded in the case of no flow within 30 s after inverting the vial. Moreover, the sol–gel phase transitions of the PLGA-PEG-PLGA aqueous solutions containing 5-fluorouracil (10 mg mL$^{-1}$) were also determined. Each data point was repeated three times and the average values were presented.

Rheology analysis
The rheology experiments of the PLGA-PEG-PLGA triblock polymer solutions in PBS (pH = 7.4) with different concentrations (15, 20, and 25 wt%) were investigated using a MCR 302 rheometer (Anton Paar, Austria). The temperature was set to increase from 10 to 70 °C at a speed of 0.5 °C per minute. The storage modulus ($G'$) and loss modulus ($G''$) were investigated under a controlled strain of 1% and a frequency of 1.0 rad s$^{-1}$. The rheology property of 25 wt% PLGA-PEG-PLGA aqueous solution containing 5-fluorouracil (10 mg mL$^{-1}$) was tested under the same conditions.
In vitro release behaviour

First, 5-Fu was completely dissolved in 20 or 25 wt% PLGA–PEG–PLGA aqueous solutions at 10 mg mL\(^{-1}\) at 4 \(^\circ\)C. 0.5 mL of the mixed solution was put into a vial with an inner diameter of 16 mm, and incubated at 37 \(^\circ\)C for 10 min to form the 5-Fu-loaded hydrogels. Then, 3 mL of phosphate buffer saline (PBS, pH = 7.4) was added into each vial as the release medium. At scheduled time intervals, 2.0 mL of the release medium containing released 5-Fu was collected, and the vials were refilled with an equal volume of fresh PBS. The amount of 5-Fu in the release medium was measured by UV spectrophotometer (UV-2401 PV, SHIMADZU) at a wavelength of 265 nm.

Cytotoxicity assay of the PLGA–PEG–PLGA copolymer

The relative cytotoxicity of the copolymer was assessed by a MTT proliferation kit (Sigma) using a L929 cell line. L929 cells were seeded in 96-well plates at 10\(^4\) cells per well in 200 \(\mu\)L of complete DMEM containing 10% fetal bovine serum, supplemented with 50 U mL\(^{-1}\) penicillin and 50 U mL\(^{-1}\) streptomycin. After incubation for 24 h, the culture medium was removed and 200 \(\mu\)L solutions of PLGA–PEG–PLGA or PEI 25K in DMEM medium were added at predetermined concentrations. After being incubated for an additional 24 h, the cells were subjected to an MTT assay and the absorbency of the solution was measured at 492 nm on a Bio-Rad 680 microplate reader. Each measurement was performed in triplicate.

In vitro gel degradation

The polymer precursor solutions (25 wt%, 0.4 mL) were placed into the vials (inner diameter = 16 mm) and incubated at 37 \(^\circ\)C to allow gel formation. After 10 min, 3 mL of PBS (pH 7.4) was added into each vial as the degradation medium. The samples were incubated at 37 \(^\circ\)C with 50 rpm. At the scheduled times, the PBS was removed, and the remaining gels were weighted. The weight loss of the gels accompanying the incubation was accurately calculated.

In vivo gel degradation and biocompatibility

Sprague-Dawley (SD) rats were used for the in vivo hydrogel degradation and biocompatibility tests. After the rats were anesthetized by diethyl ether, 0.4 mL of 25 wt% polymer precursor solution was subcutaneously injected into the back area of the rats using a 21-gauge needle. The rats were sacrificed and the images of the gels were recorded at scheduled times. The skin tissues near the gels were cut off and stained with hematoxylin and eosin (H&E) to examine the inflammatory responses of the rats.

In vivo anti-adhesion test

Animal model. All operations on the animals were carried out in accordance with the policies of Jilin University School of Medicine. For in vivo anti-adhesion test, 24 male SD rats weighing 250–300 g were anesthetized by intraperitoneal injection of chloral hydrate (30.0 mg kg\(^{-1}\)). The skin near the right Achilles tendon was shaved and sterilized by alcohol. A midline incision about 3 cm was made from calcaneal bone and over the right Achilles tendon. After isolating the surrounding tissue, the tendon was cut with a scalpel approximately 5 mm from the calcaneal bone and the tendon was then sutured using a Kessler stitch with 6-0 silk suture (PGA Resorba®, Resorba, Nürnberg, Germany). Then, the rats were divided into 3 groups randomly as follows: Group I as the control group without additional treatment, Group II that were treated by injection of 150 \(\mu\)L of the PLGA–PEG–PLGA hydrogels without 5-Fu around the repaired tendons, and Group III that were treated with 150 \(\mu\)L of 5-Fu-loaded PLGA–PEG–PLGA hydrogels (concentration of 5-Fu = 10 mg mL\(^{-1}\)). The thicknesses of the hydrogels formed around the tendons were generally about 1–2 mm. All procedures were operated under sterile conditions in an animal operating room.

Macroscopic evaluations. At 3 weeks after surgery, we examined the surgical sites to check for any sign of inflammation and ulcer before the rats were killed. The breadth and severity of Achilles tendon adhesion were scored. The adhesion score was recorded into 1 to 5 grades, as it was based on surgical findings. The adhesion score was evaluated by an observer who was blind to assess the experimental groups.

Histological analyses. All Achilles tendons were collected after sacrificing the rats and fixed in 4% (W/V) PBS-buffered paraformaldehyde. After the specimens were embedded in paraffin, 5 \(\mu\)m-thick sagittal and longitudinal sections were obtained and stained with H&E and Masson’s trichrome. All images were recorded by a light microscope.

Statistical analyses. Statistical significances were analyzed by SPSS (Version 13.0, Chicago, IL, USA). Significant differences were reported as the \(p\) value less than 0.05. The data were presented as means ± standard deviations.

Results and discussion

Synthesis and characterization of the PLGA–PEG–PLGA copolymer

The triblock copolymer of PLGA–PEG–PLGA was prepared through the ring-opening polymerization of \(\text{\textcopyright}_{15} \text{Lactide} \) (LA) and glycolide (GA) using PEG (\(M_n = 1500\)) as the macroinitiator and \(\text{Sn(Oct)}_2\) as the catalyst.\(^{22,23}\) The structure and molecular weight of the resulting copolymer were evaluated by \(^1\text{H NMR}\) and GPC. Typical peaks assigned to PLGA–PEG–PLGA were observed in the \(^1\text{H NMR}\) spectrum, as shown in Fig. 1. Based on the \(^1\text{H NMR}\) result, the molecular weight of the triblock copolymer was 4600 and the LA/GA molar ratio was 12 : 1. The molecular weight and composition of the resulting copolymer are listed in Table 1. The aforementioned results indicated that the PLGA–PEG–PLGA triblock copolymer was synthesized successfully.

Sol-gel phase transitions and rheological properties of the triblock copolymer solutions

The PLGA–PEG–PLGA aqueous solutions revealed a sol–gel phase transition with increasing temperature, as shown in Fig. 2A. When the temperature increased from 5 to 70 \(^\circ\)C, the
solutions displayed three physical states: sol, gel, and sol (precipitation), as shown in the inserted images of Fig. 2A. When the polymer concentration increased from 15 to 25 wt%, the sol-to-gel transition temperature decreased, whereas the gel-to-precipitation transition temperature increased, leading to the widening of the gel window. Furthermore, the triblock copolymer solution containing 5-fluorouracil (5-Fu) also exhibited a sol–gel–precipitation phase transition with increasing temperature, as shown in Fig. 2A. It was found that the addition of 5-Fu resulted in a slight shift (~1 °C) of the sol–gel phase diagram.

The mechanism of the sol–gel–precipitation phase transition may be attributed to the following explanation: at low temperatures, the triblock copolymers could self-assemble into micelles in aqueous solution which consist of hydrophobic PLGA cores and hydrophilic PEG shells. As the temperature increases to the sol–gel transition temperature, inter-micellar aggregation is formed due to the partial dehydration of the PEG shells and the increase in the hydrophobic interaction of the PLGA chains.\textsuperscript{32,34,35} Moreover, when the temperature is further increased, the micelles shrink, obviously caused by the significant dehydration of the PEG shell, leading to the precipitation of the triblock copolymer.

Furthermore, the temperature-dependent rheological properties of the copolymer aqueous solutions, with or without 5-Fu, was investigated. As shown in Fig. 2B, the storage moduli of the samples exhibited an abrupt increase, as the temperature was enhanced from 10 °C. It was found that the onset of the increase in storage modulus ranged from 28 to 32 °C depending on the polymer concentration, with the maxima at around 36 °C. The increase in the polymer concentration led to a reduction in the onset temperature, which is consistent with the sol–gel transition temperature observed by the tube inverting method. Additionally, the addition of 5-Fu showed no obvious effect on the temperature region for the gel formation but caused a slight reduction in the storage modulus, suggesting that the drug may have some effect on thermo-induced micellar aggregation (Fig. 2B).

**In vitro release of 5-fluorouracil from the hydrogels**

The *in vitro* release profiles of 5-Fu from the 20 and 25 wt% PLGA–PEG–PLGA hydrogels are shown in Fig. 3. It was shown

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**Table 1** Characterization of the PLGA–PEG–PLGA triblock copolymer

<table>
<thead>
<tr>
<th>Entry</th>
<th>$M_n$ (^a)</th>
<th>PLGA/PEG (wt/wt) (^a)</th>
<th>LA/GA (mol mol(^{-1})) (^a)</th>
<th>$M_n$ (^b)</th>
<th>PDI (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGA–PEG–PLGA</td>
<td>1550–1500–1550</td>
<td>2.1/1</td>
<td>12/1</td>
<td>9680</td>
<td>1.18</td>
</tr>
</tbody>
</table>

\(^a\) Determined by \(^1\)H NMR. \(^b\) Determined by GPC.
that the sustained release of 5-Fu from the hydrogels last for up to 7 days. The increase in the polymer concentration resulted in a reduction of the drug release rate. Two kinds of mechanism may be involved in the drug release from the hydrogels, including a diffusion-controlled manner in the initial stage and the combined mechanism of diffusion and degradation in the later stage. The release profiles of this study are in agreement with the results in the literature.

**In vitro and in vivo degradation and biocompatibility**

As the important factors for biomedical applications of polymer materials, the degradation and biocompatibility of the PLGA–PEG–PLGA hydrogels were investigated *in vitro* and *in vivo*. When incubated in PBS at 37 °C, about 90% of the PLGA–PEG–PLGA hydrogels degraded in 27 days (Fig. 4). The *in vivo* gel formation and degradation were also evaluated in SD rats. The triblock copolymer solutions (23 wt%, 0.4 mL per rat) were subcutaneously injected into the dorsum of the rats. At 15 min post-injection, the gels were formed *in situ* subcutaneously (Fig. 5). It was found that the hydrogels degraded gradually in the subcutaneous layer during the following 3 weeks and were completely eliminated after 4 weeks (Fig. 5). This indicated that the hydrogels displayed a faster degradation rate in the subcutaneous layer of rats than in PBS *in vitro*. The reasons for the faster degradation rate *in vivo* are likely attributed to several aspects, including cellular and enzymatic effects in the subcutaneous layer and only one gel surface exposure to the

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**Fig. 3** Release profiles of 5-Fu from the drug-loaded PLGA–PEG–PLGA hydrogels with the polymer concentrations of 20 and 25 wt%, respectively.

**Fig. 4** *In vitro* mass loss profiles for the PLGA–PEG–PLGA hydrogels with polymer concentrations of 20 and 25 wt%.

**Fig. 5** *In vivo* gel formation and degradation of the PLGA–PEG–PLGA hydrogels (0.4 mL, 25 wt%) after the subcutaneous injection into rats. Images around the gels were taken at 15 min (0 day), 7, 14 and 28 days after the injection.

**Fig. 6** *In vitro* cytotoxicities of the copolymer with different concentrations (0.063–1.0 g L⁻¹) to L929 cells with PEI 25K as a positive control. Data are presented as mean ± standard deviation (n = 3).
release media in the in vitro degradation test. The above results indicated that the triblock copolymer hydrogels could degrade in vitro and in vivo.

To investigate the in vitro cyto-compatibility of the triblock copolymer, an MTT assay was performed to evaluate the cytotoxicity of the materials against fibroblast L929 cells. The L929 cells were incubated with the copolymers at different concentrations (0.063–1.0 g L⁻¹) for 24 h and PEI 25K was used as a positive control. The cell viabilities are shown in Fig. 6. It was observed that the L929 cells treated with the copolymer remained more than 90% viable at all concentrations up to 1.0 g L⁻¹. The result indicated a good cyto-compatibility of the triblock copolymer.

To further evaluate the in vivo biocompatibility of the hydrogels, the inflammatory response of the host tissues to the hydrogels was analyzed after subcutaneous injection of the hydrogels into rats. The tissue surrounding the hydrogels were dissected and H&E-stained at the predetermined time. As shown in Fig. 7, at 7 days post-injection, an increasing number of neutrophils were observed, indicating an acute inflammation. Nevertheless, neutrophils were reduced and the acute inflammatory turned into a chronic inflammation gradually with the degradation of the hydrogels. As the hydrogels disappeared after 28 days following injection, the inflammation was nearly eliminated and the surrounding tissue returned to normal. The results suggested that the hydrogels were acceptable to the animal body and exhibited a good biocompatibility in vivo.

In vivo evaluation of Achilles tendon adhesion prevention

The 5-Fu-loaded hydrogel was used to evaluate the potential application for adhesion prevention in an Achilles tendon repair model of rats. Fig. 8 shows the procedure of the anti-adhesion test in vivo. First, the Achilles tendon was exposed and the tendon was cut off with a scalpel. Then, it was sutured by using a Kessler stitch. The 5-Fu-loaded hydrogels (150 μL) with 10 mg mL⁻¹ of 5-Fu was injected to the surrounding of the repaired tendon after stitching and the incision was closed. As shown in Fig. 8, the hydrogels completely covered the Achilles tendon, which facilitates the complete distribution of the drug-loaded hydrogels.

At 3 weeks after surgery, the rats were sacrificed and there were no infections and ulcers for all the rats during the experimental period. As shown in Fig. 9, a large range of adhesion formed around the Achilles tendon in the control group (Fig. 9A and B) and the adhesion score was recorded as 5 (Fig. 10). The Achilles tendon treated with hydrogels only showed a smaller area of adhesion compared to the control group, and it could be separated by a little harder blunt dissection (Fig. 9C and D). The average adhesion score of the group treated with hydrogel only was ~3. Notably, the lowest adhesion score was found in the group treated with 5-Fu-loaded hydrogels (Fig. 10). No adhesion...
or an extremely minor adhesion was observed and the adhesion could be easy to be separated (Fig. 9E and F). Thus, the results indicated that the 5-Fu-loaded hydrogels displayed significantly reduced adhesion formation, compared to either the untreated group or the group treated only with hydrogel.

The microscopic evaluation was performed by H&E and Masson’s trichrome staining to observe the inflammation and collagen regeneration (Fig. 11). As shown in Fig. 11A and C, a large amount of inflammatory cells could be seen around the repaired tendons in the control group and the group treated only with hydrogel. In contrast, very few inflammatory cells were found in the group treated with 5-Fu-loaded hydrogels (Fig. 11E). This suggested that the release of 5-fluorouracil led to a reduction in inflammatory responses. In addition, the Masson’s trichrome staining displayed that the quantity of regenerated collagen in the group treated with 5-Fu-loaded hydrogels was less than the control group and the group treated only with hydrogel. Moreover, it was observed that the regenerated collagen in the group treated with 5-Fu-loaded hydrogels showed more organized arrangement (Fig. 11B, D and F).

The process of tendon healing usually includes extrinsic and intrinsic healing processes. The extrinsic healing plays a key role in the occurrence of tendon adhesion. Thus, how to inhibit the extrinsic healing is crucial for the prevention of post-operative tendon adhesion. In this study, the adhesion score of the group treated with the hydrogels without 5-fluorouracil was lower than that of the untreated group ($p < 0.05$). This might be due to the fact that the PLGA–PEG–PLGA hydrogels acted as physical barriers and reduced the invasive growing of the extrinsic fibroblast into the tendon. However, the pathological sections of this group showed that inflammatory cells and proliferative fibroblasts were comparable to the control group (Fig. 11B and D). In addition to the physical barriers, the combination with anti-adhesion agents may lead to enhanced anti-adhesion efficacy. As a kind of anti-metabolism and anti-tumor drug, 5-fluorouracil can inhibit cell proliferation by the suppression of DNA replication, and it was used to prevent tendon adhesions after surgery.\textsuperscript{25,26,28,29} In this examination, the group treated with 5-Fu-loaded hydrogels showed a significant

![Images of the adhesion evaluation of rat Achilles tendon for different groups.](image)

Fig. 9 Images of the adhesion evaluation of rat Achilles tendon for different groups. (A and B) Control group: without treatment. Black arrow indicates the Achilles tendon adhesions. (C and D) Group treated with the hydrogel without 5-fluorouracil. (E and F) Group treated with 5-Fu-loaded hydrogel.

![Adhesion scores of the experimental groups.](image)

Fig. 10 Adhesion scores of the experimental groups: (A) the average of adhesion scores and (B) distribution of adhesion scores. Data are presented as mean ± SD ($n = 8$; *$p < 0.05$).
In this study, we present an investigation of the application of 5-fluorouracil-loaded thermosensitive hydrogels as a physical barrier for preventing postoperative tendon adhesion. The thermosensitive PLGA–PEG–PLGA hydrogels, with or without, 5-fluorouracil showed a thermo-induced sol–gel–precipitation phase transition and displayed maximum storage moduli at around physiological temperature. The hydrogels degraded in 4 weeks after being injected in the subcutaneous layer of rats, and exhibited acceptable biocompatibility in vivo. After treating the sutured Achilles tendon model of rats with the hydrogels, the group treated with 5-fluorouracil-loaded hydrogels showed significantly reduced adhesion formation, compared to either the untreated group or the group treated only with the hydrogels. Hence, this study suggested that treatment with 5-fluorouracil-loaded PLGA–PEG–PLGA hydrogels may hold potential as an efficient approach for preventing postoperative tendon adhesion.

Conclusions

In this study, we present an investigation of the application of 5-fluorouracil-loaded thermosensitive hydrogels as a physical barrier for preventing postoperative tendon adhesion. The thermosensitive PLGA–PEG–PLGA hydrogels, with or without, 5-fluorouracil showed a thermo-induced sol–gel–precipitation phase transition and displayed maximum storage moduli at around physiological temperature. The hydrogels degraded in 4 weeks after being injected in the subcutaneous layer of rats, and exhibited acceptable biocompatibility in vivo. After treating the sutured Achilles tendon model of rats with the hydrogels, the group treated with 5-fluorouracil-loaded hydrogels showed significantly reduced adhesion formation, compared to either the untreated group or the group treated only with the hydrogels. Hence, this study suggested that treatment with 5-fluorouracil-loaded PLGA–PEG–PLGA hydrogels may hold potential as an efficient approach for preventing postoperative tendon adhesion.

Notes and references