Encapsulation of cell-adhesive RGD peptides into a polymeric physical hydrogel to prevent postoperative tissue adhesion

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Abstract: Peptides containing the sequence of arginine-glycine-aspartate (RGD), a famous adhesion moiety, can specifically conjugate integrins in cell membranes, and are usually applied to enhance cell adhesion after linking to solid substrates in tissue engineering or to nanoparticles in targeting delivery. This paper reveals, however, that free RGD peptides can assist in preventing tissue adhesion by blocking focal adhesion between cells and surfaces of barrier devices. In order to avoid a rapid peptide loss after straightforward injection of a peptide solution, we employed a thermosensitive injectable hydrogel composed of a biodegradable block copolymer poly(e-caprolactone-co-lactide)-poly(ethylene glycol)-poly(e-caprolactone-co-lactide) (PCLA–PEG–PCLA) to encapsulate peptides cyclo(RGDfK). A sustainable release for one week was achieved in vitro. The rabbit model of sidewall defect and bowel abrasion was selected to examine the in vivo anti-adhesion efficacy. It reveals a significant reduction of postoperative peritoneal adhesion in the group of RGD-loaded PCLA–PEG–PCLA hydrogels. We interpret this excellent efficacy by the combination of two effects: first, our hydrogel affords a physical barrier to prevent adhesion between injured abdominal wall and cecum; second, the RGD molecules as integrin blockers released from the hydrogel assist the anti-adhesion. © 2012 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 100B: 1599–1609, 2012.

Key Words: postoperative adhesion, thermosensitive hydrogel, adhesion prevention, RGD peptide, controlled release


INTRODUCTION

Postoperative adhesions are well-recognized serious complications in all surgical subspecialties.1,2 Especially for the abdominal adhesion, the occurrence was reported as high as 80% in abdominal and pelvic surgery,3 leading to pelvic pain, bowel obstruction, and even infertility in women.4 Antiadhesion is thus very important.

In general, there are two anti-adhesion strategies: (1) pharmaceutical treatments5,6 and (2) barrier-based devices.7–10 To prevent tissue adhesion, a number of bioactive substances such as anti-inflammatory agents11,12 and fibrinolytic agents5,13 have been tried. Pharmaceutical treatments alone do not prevent adhesions very effectively due to the rapid loss of drugs from the peritoneum, and thus sustained release is required. On the other hand, barrier devices, such as polymer films and hydrogels,14–21 play important roles in antiadhesion. The above two strategies could be used simultaneously.11,13,22

Herein we also use a combinatory method to significantly reduce the formation of postoperative tissue adhesion by integrating both a barrier-based device and a pharmaceutical treatment. We employed a mixture of a block copolymer poly(e-caprolactone-co-lactide)-poly(ethylene glycol)-poly(e-caprolactone-co-lactide) (PCLA–PEG–PCLA) and a cell-adhesive peptide containing the sequence of arginine-glycine-aspartate (RGD). The polymeric aqueous solution could be, free of any chemical reaction, physically gelled at body temperature. Similar thermogels have been extensively investigated as injectable medical materials23–25 mainly for controlled release carriers,26–28 partly for tissue engineering29,30 and recently for antiadhesion.21,31

The RGD peptide is a star molecule in biomaterials and cell biology.32–39 RGD-containing peptides are recognized as "cell-adhesive" molecules due to their specific conjugation with integrins in cell membranes to form focal adhesions.33,34,37,38,40,41 Biomaterial scientists usually use the RGD-containing peptides to promote cell adhesion after immobilizing them into solid substrates.32,35,39 or semisolid hydrogels,42–47 In Pharmaceutics, RGD peptides are also used to prepare integrin-targeting carriers after linking...
them into nanoparticles for drug delivery, gene delivery, and image enhancement.

In our previous report, we confirmed that the chemical binding of RGD into block copolymer PCLA–PEG–PCLA enhanced in vitro cell adhesion on the corresponding hydrogels in order to develop potential injectable tissue engineering materials. In this work, we anticipated that the unbound RGD might be beneficial for preventing tissue adhesion. Since a straightforward injection of a RGD solution might lead to rapid clearance of the agent from the body site, we tried to employ our PCLA–PEG–PCLA hydrogel to encapsulate RGD peptides. The principle is schematically presented in Figure 1. The present paper is aimed to examine the feasibility of the sustained release of RGD from the thermogel and also the in vivo efficacy of preventing postoperative adhesions of the RGD-encapsulated hydrogels.

MATERIALS AND METHODS
Materials
Poly(ethylene glycol) (molecular weight MW 1500), stannous octoate (95 %), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich; ε-caprolactone (CL) (95%) was bought from Acros and distilled over calcium hydride; d,l-lactide (LA) was from Purac and used as received; fetal bovine serum (FBS) and minimum essential medium alpha (MEMα) were from Gibco; trifluoroacetic acid (TFA), dichloromethane (DCM), dimethylsulfoxide (DMSO) and other solvents were

![Figure 1](Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.)
purchased from Shanghai Chemical Reagents Co. All other chemicals were of reagent grade and used as received.

Animals
White New Zealand rabbits (male, 2.5 ± 0.2 kg) supplied by the Experimental Animal Center of Fudan University were acclimatized at a temperature of 25 ± 2°C and a relative humidity of 70 ± 5% under natural light/dark conditions at least for one week before the experiment. All the animal experiments adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985), and were approved by Animal Care and Use Committee of Zhongshan Hospital, Fudan University.

Synthesis of cyclic peptide
The peptide cyclo(-RGDK-) was synthesized via Fmoc-protected solid phase peptide synthesis strategy as described in our previous report. Here, ‘F’ refers to o-phenylalanine, and other four residues are of an L configuration. In brief, the amino acids Gly, Arg, Lys, Phe, and Asp protected by 9-fluoromethoxy carbonyl (Fmoc) were coupled one by one to form linear peptides on the 2-chlorotriyl chloride (CTC) resin. For elongation of peptide by each amino acid, the corresponding amino acids were added to the resin together with five folds of the solution of O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) and N,N-diisopropylethylamine (DIPEA); after reacting for 30 min, Fmoc groups were cleaved by 20% (v/v) piperidine in N,N-dimethylformamide (DMF) for 10 min. The linear peptide was then cleaved from the resin by 5% (v/v) trifluoroacetic acid (TFA) in DCM for 1 h. Then peptide cyclization was carried out in the solvent by (benzotriazol-1-yl-oxycarbonyl)tritylroldinophosphonium hexafluorophosphate (PyBop) and DIPEA for 24 h. All the protecting groups were removed by using 95% (v/v) TFA/H2O for 2 h. The de-protected cyclic peptide was purified by preparative reversed-phase high performance liquid chromatography (RP-HPLC) on Waters 2545 Alliance system. The product was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, Applied Biosystems).

Synthesis of triblock copolymer
The PCLA–PEG–PCLA triblock copolymer was prepared following typical ring-opening polymerization of LA and CL using PEG as initiator and stannous octoate as catalyst. Briefly, PEG (5.0 g) was heated at 130°C under vacuum to eliminate the trace amount of water, and then CL (5.8 g) and LA (5.8 g) together with stannous octoate (0.2 wt % of monomers) were added. The mixture was heated under argon protection at 130°C for 12 h. The copolymer was purified by dissolving-precipitation in water, and the precipitate was then lyophilized to collect the final product.

1H-NMR was used to characterize the chemical structure of the block copolymer. The methylene protons in the ethylene glycol units at 3.65 ppm, methine protons of lactic acid units at 5.10 ppm, and α-methylene protons in the caprolactone units around 2.35 ppm were used for the determination of number-average molecular weight $M_n$ of the PCLA–PEG–PCLA triblock copolymer with the predetermined MW of the central PEG block (1500) as afforded by Sigma-Aldrich.

Gel permeation chromatography (GPC, Agilent1100) equipped with a differential refractometer as detector was used to obtain MW and its distribution of block copolymers. The unimodal GPC trace with a low polydispersity index confirmed the formation of triblock copolymers.

In vitro release of peptides from polymer hydrogels
Peptides were dissolved in the PCLA–PEG–PCLA triblock copolymer solution (20 wt % in the normal saline) at concentrations of 1, 5, or 10 mM, and polymer solutions without peptides were set as blank controls. After the addition of 0.5 mL of each sample solution ($n = 4$), the tubes were heated to 37°C in a water bath to form hydrogels. Then 10 mL of pre-heated phosphate buffered saline (PBS, pH 7.4) was added to each tube. The tubes were then sealed to prevent water evaporation and incubated in a shaking bath at 37°C with 50 stroke/min.

The device for in vitro release experiments is shown in Figure S1 in Supporting Information. At predetermined time points, half of the buffer was taken out of the tubes, and replaced by a fresh one to keep the sink condition. RGD peptides in the release medium was treated by the modified Sakaguchi’s reaction, and the reactants were then detected by UV-vis spectrum at 505 nm. The released medium of the drug-free hydrogel at each associated time (also experiencing the modified Sakaguchi’s reaction) was taken as the blank control. The amount of RGD peptides released from the hydrogel was obtained from the standard curve of cyclo(-RGDK-) solutions as shown in Supporting Information Figure S2. The accumulated release profiles were then obtained.

Observations of sol-gel transition
The sol–gel transition of the copolymer solutions (20 wt % dissolved in the normal saline, with or without peptide) were investigated in a strain-controlled rheometer (ARES Rheometric Scientific) using a Couette cell. Cold polymeric solutions were transferred into the Couette cell and carefully overlaid with a thin layer of low-viscosity silicone oil to minimize solvent evaporation. Temperature was controlled with an accuracy of ± 0.05°C by an environment controller (Neslab, RT 130). The heating rate was 0.5°C/min, and the angular frequency $\omega$ 10 rad/s.

The phase transition was also roughly estimated by vial-inverting approach: 0.5 mL of the polymer solution (20 wt % in the normal saline) was added to a 2 mL vial and stored at 4°C for 12 h before test. The vial was then immersed in a water bath and allowed to reach equilibrium. The sample was regarded as a “gel” when no visual flow occurred within 30 second by inverting the vial.

In vitro viability of cells treated by free RGD peptides
The viability evaluation of cells treated by dissolved RGD before or after adhering to the substrate was determined by...
the MTT assay of the cell line MC-3T3. Cells were seeded in 24-well plates at $4 \times 10^4$ cells per well. To study the effect of RGD peptides, cells were divided into three groups which abbreviated as "Pre", "Post," and "Blank". The time maps are schematically presented in Supporting Information Figure S3. For the group of "Pre," cell suspensions were pretreated with a 5 mM peptide solution of cyclo(-RGDfK-) in MEMa at 37°C for 30 min, while for the other two groups, MEMa was used alone; then culture medium was replaced with a fresh one (MEMa + 10% FBS); for the group of "Post", the culture medium was, after incubated for 12 h (37°C, 5% CO2), replaced by a 5 mM RGD solution of MEMa and incubated for 30 min, while the other two groups received a culture medium without RGD for 30 min, then the culture medium was again replaced by a fresh one (MEMa + 10% FBS). After a following 24-h incubation, the MTT assay was tested: cells cultured in 1000 μL of the MEMa medium per well received 100 μL of MTT (5 mg/mL), followed by an incubation at 37°C for 4 h, then the MTT-containing medium was replaced by 200 μL of DMSO, and the absorbance of the samples was measured at 492 nm. The viability of cells from the group of "Blank" was set as 100%.

In vivo evaluation of intestinal adhesion-prevention efficacy

A rabbit model of sidewall defect and bowel abrasion was used to evaluate the prevention efficacy of postoperative adhesions. After general anesthesia by ketamine hydrochloride and xylazine hydrochloride (1:1 mixture, 1.0 mL/kg via muscle injection), peritoneal intestinal adhesions were induced with a defect of $4 \times 3$ cm$^2$ on the right lateral abdominal wall and by abrading corresponding cecal haustra for 50 strokes with a surgical brush. The rabbits were randomly divided into four groups. In the group of "Gel + RGD," 4 mL of PCLA–PEG–PCLA solution containing cyclo(-RGDfK-) was injected to cover the defects in each animal (peptide concentration: 5 mM, polymer concentration: 20 wt %). The gelation occurred rapidly in situ. The peritoneum and abdominal wall were closed with 3-0 silk sutures, and the skin was closed with 4-0 silk sutures. In the group of "RGD," the same volume of peptide solution (5 mM in the normal saline) was used. In the group of "Saline," rabbit defects were received 4 mL of the normal saline per animal, which was set as another control.

Seven days after surgery, all of the rabbits were euthanized to examine postoperative adhesions. Planimetry was used to determine the area of the sidewall injury where intestinal adhesion had developed. The tenacity of the intestinal adhesion was rated as follows: Score 0, no adhesion; Score 1, mild, easily separable intestinal adhesion; Score 2, moderate intestinal adhesion, separable by blunt dissection; and Score 3, severe intestinal adhesion requiring sharp dissection to separate. The scores were taken by a double-blind process. Specimens were fixed in 10% formalin, embedded in
paraffin, sectioned, and stained with the hematoxylin and eosin (HE) staining for histological examinations.

Since the score does not obey the Gaussian distribution, our statistical analysis of the tenacity evaluation was made via the Kruskal-Wallis test, while a Student t-test was used for the statistical evaluation of adhesion areas.

RESULTS

Synthesis of block copolymer and peptide

The triblock copolymer PCLA–PEG–PCLA was synthesized via ring-opening polymerization of CL and LA initiated by the terminal hydroxy groups of PEG (MW 1500). The $M_n$ of the copolymer was 4900 (1700-1500-1700) as determined by $^1$H NMR. The polydispersity index ($M_w/M_n$) was 1.23 as characterized by GPC. The block copolymer was then dissolved in the normal saline at room temperature to form a 20 wt % solution.

We used Fmoc-protected solid phase peptide synthesis to obtain cyclo(-RGDfK-), and then purified it by RP-HPLC. In the MALDI-TOF measurements, the mass-charge ratio of the molecular peak $m/z = 605.1$, while the theoretical $m/z$ considering one extra proton in the peptide is 604.7. The experimental value was thus in a reasonable error range.

Chemical structures of the peptide and block copolymer are presented in Figure 1(C).

In vitro viability of adhered and unadhered cells treated by free RGD peptides

The anchorage-dependent cells (MC-3T3) were treated with RGD before ("Pre") or after ("Post") adhering to the substrate. The time maps are presented in Supporting Information Figure S3. Compared to the blank control, viability of cells in the "Pre" group decreased about 60 %; in the "Post" group, cell viability decreased only a little, as shown in Figure 2. So the free RGD peptides can prevent cell adhesion of unadhered cells by saturation of conjugation sites of integrins, while the adhered cells are less sensitive to RGD peptides because many integrins in cell membranes have already combined to the ligands on the substrate.

Sol–gel transition of the peptide-loaded PCLA–PEG–PCLA hydrogel

We confirmed that the peptide did not hamper the physical gelation of the block copolymer PCLA–PEG–PCLA in water. The sol–gel transition of 20 wt % polymer solution (in the normal saline) containing 5 mM of cyclo(-RGDfK-) was qualitatively observed through the vial-inverting approach. The 20 wt % polymer solution was a flowable sol at room temperature, but turned to be a gel at body temperature (37 °C), as shown in Figure 3(A).

We further quantitatively detected the sol-gel transition in a strain-controlled rheometer. The storage modulus ($G'$)
increased for about four orders of magnitude when the sample was heated from the room temperature to body temperature, along with the increase of the loss modulus ($G''$). The sol-gel transition temperature $T_{gel}$ with respect to $G' = G''$ is about 30°C according to Figure 3(B), which is in good agreement with the result of our vial-inverting test.

FIGURE 5. Photographs of the animal experiments of postoperative adhesions in a rabbit model with peritoneal wall and cecum defects. Upper row: Left: a defect (4 × 3 cm²) comprising the peritoneum and a layer of muscle (~1 mm thick) was generated starting 1 cm from the midline of peritoneal wall; Right: the corresponding site on cecum was abraded until bleeding by a surgical brush. Arrows assist displaying the defect boundaries. Middle and lower rows: photographs of gross observations of the efficacy of prevention adhesion of defected tissues after 7 days. Score 0: no adhesion; score 1: mild, easily separable intestinal adhesion; score 2: moderate intestinal adhesion, separable by blunt dissection; score 3: severe intestinal adhesion, non-separable unless using sharp dissection. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
Figure 6. Adhesion tenacity scores of rabbits treated with cyclo(-RGDFK-), released from PCLA-PEG-PCLA hydrogel (named as “Gel + RGD”), the hydrogel (“Gel”), RGD solution (“RGD”), or the normal saline alone (“Saline”). The p values are presented in Supporting Information Table S2. The asterisks denote significant difference compared to the saline group (**: p < 0.05; ***: p < 0.01).

Previous studies have revealed that the sol–gel transition of this polymer solution is readily influenced by some additives such as salts and even homopolymers.53–55 Rheological measurement results of the polymer solutions with or without RGD peptides are presented in Figure 3B and Supporting Information Figure S4, respectively. The comparison illustrates that $G’$, $G’’$, and $T_{gel}$ were not obviously influenced by RGD peptides. This sol–gel transition of the RGD-containing polymer solution enables its potential as an injectable material, and such an injectable physical hydrogel is rather convenient for surgery operation.

**In vitro release of encapsulated peptides from hydrogels**

Since RGD is a water-soluble small molecular agent, it is hard to predict whether or not a hydrogel could release it in a sustainable manner. The in vitro release of cyclo(-RGDFK-) from our polymer hydrogel was carried out in test tubes under a mimetic physiological condition. The release profiles of RGD peptide are shown in Figure 4. A sustainable release was found, which lasted for one week with a cumulative release of about 80%.

In vitro drug release via diffusion can be described by Higuchi equation, which is written as

$$Q = k\sqrt{t}(Q < 0.6)$$

Here, $Q$ is the fraction of drug release at time $t$, and $k$ is a constant related to diffusivity and also some structural and geometry parameters. Just as shown in Table 1, all of the three samples exhibit high squared correlation coefficients of about 0.98. So, the release of hydrophilic peptides should be mainly contributed by molecular diffusion.

**In vivo evaluation of antiadhesion efficacy**

The rabbit model of sidewall defect and bowel abrasion was used to induce peritoneal intestinal adhesions. The defects created in the abdominal wall and cecum are shown in Figure 5. A polymer solution (20 wt % in the normal saline) containing cyclo(-RGDFK-) (5 mM) was injected onto the defects (marked as “Gel + RGD”) to prevent tissue adhesion. A polymer solution (“Gel”), a peptide solution (“RGD”) and the normal saline alone (“Saline”) were examined as well.

Seven days after surgery, all the animals were euthanized for evaluating the intestinal adhesions. The adhesion tenacity was scored, as demonstrated in Figure 5. The results in Figure 6 show the lowest mean adhesion score of rabbits receiving “Gel + RGD,” indicating the best anti-adhesion efficacy of this group.

We also measured the area of the adhered defects, with the results shown in Figure 7. Although a positive correlation exists between the adhesion area and tenacity as usual, these two parameters have different emphases and are not equivalent to each other. The adhesion area refers to extension of adhesion, while the tenacity estimated by scores is more correlated to the micro-structure of adhesion tissues. The average adhesion area in the group of “RGD” alone decreased for about 1.5 cm$^2$ while its adhesion tenacity score reduced just from 2.5 to 2.3. It demonstrated that the efficacy of adhesion prevention by direct injecting an RGD solution was limited, probably due to the rapid clearance of peptides in vivo. A combinatory effect on adhesion prevention by free-dissolved RGD molecules and hydrogels was found in the group of “Gel + RGD”.

The histological observations of the adhesion sites were further carried out. In the group of “Gel + RGD,” the injury site was covered with new mesothelium, and adhesion was well prevented resulting in the histological structure similar to that of the normal tissue. In contrast, most abdominal wall defects treated with “RGD” or “Saline” exhibited a granulated and loose tissue between cecum and abdominal wall, and a large number of inflammatory cells were observed, as presented in Figure 8. It seems also necessary to note that we have not found any noticeable tissue...
necrosis in “RGD,” “Gel,” or “Gel + RGD” group as well as “Saline” group.

DISCUSSION
Postoperative adhesions are a classic and frequent problem, especially in the peritoneal surgery. Adherent tissues are most likely to form within 7–10 days after surgery. One of the adhesion mechanisms is summarized as follows: in the first phase for the initial several hours after surgery, coagulations such as platelets and fibrinous exudates are concentrated on the wound site, and a large number of inflammatory cells such as neutrophils and a succeeding of...
macrophages are migrated from the neighboring tissues or the peritoneal fluid; in the second stage by the fourth day of injury, the inflammatory cells are still dominated by macrophages, although some of deposited fibrin are dissolved by plasminogen, and fibroblasts get to present in fibrin bridges, initiating deposition of collagen in the adhesion band; then, by the fifth day after surgery, the initial fibrin-composed matrix is gradually replaced by collagen, and the number of fibroblasts are greatly increased, resulting in a significant increase of adhesion tenacity.

As antiadhesion is concerned, implantations of biomedical barrier devices are considered as a useful strategy. Nevertheless, still many cases failed, and sometimes the tissue adhesions developed even on the surfaces of the barriers. Another strategy comes from pharmaceutical treatments. But the rapid drug clearance from peritoneum limits the efficacy of this strategy to a large extent. In this study, we combined the pharmaceutical and barrier-based strategies to prevent postoperative adhesions by using the RGD-encapsulated PCLA–PEG–PCLA thermogel. Mixing RGD into the polymeric aqueous solution did not hamper injectability of the sol and the forthcoming physical gelation after injection. Despite low molecular weight and water solubility, a sustained release was achieved for about one week. The timing fits well with the adhesion formation process, as the most efficient therapeutic window to prevent adhesion formation lies within the first 5-7 days after surgery. The injectability of the material brings with much convenience in operation. The gelation happens free of any chemical reaction. Moreover, compared to other drug carriers such as polymer beads or microgels, the in-situ gelation of the physical hydrogel promises an almost 100 % drug encapsulation, which is another advantage of this thermogelling system on loading of the precious peptides.

The peptide cyclo(-RGDfK-) was synthesized in vitro and used in vivo. We selected the cyclic form, because the cyclic structure provides a higher combining efficiency and better enzyme stability than the linear one. The free-dissolved RGD peptides were confirmed to inhibit cell adhesion in vitro, if the integrins of a cell were saturated by free peptides before the cell attached to a substrate. The integrin-saturated cells exhibited lower adhesion to barrier surfaces or other tissues, as demonstrated in our in vitro cell adhesion experiments in Figure 2. Therefore, the prevention of adhesion formation by the local release of free cyclo(-RGDfK-) might be attributed to conjugation of RGD molecules to integrins of inflammatory cells.

The RGD sequence was found to influence the process of inflammation diseases with cells such as T lymphocytes involved, and the αvβ3 integrin was reported to regulate macrophage inflammatory responses. On the basis of the literature relevant to prevention of adhesions and effects of RGD, the released RGD in our animal experiments might inhibit integrins to allow the T lymphocytes and macrophages to escape from the inflammatory site. A lower adhesion rate of inflammatory cells might further reduce collagen secretion and then enhance the anti-adhesion efficacy. As recently reviewed by Brochhausen et al., "Adhesion formation is the result of complex interactions of multiple cellular and humoral factors during wound healing of serous membranes. However, the exact pathomechanisms are still poorly understood." The eventual mechanism of the RGD effects on in vivo antiadhesion in our experiments is open as well. The absorption and pharmacokinetics of the molecules are also expected to be explored.

The excellent in vivo efficacy of the group "Gel + RGD" comes from both RGD and the physical hydrogel. One block of this copolymer, PEG, might, as a famous nonfouling molecule, contribute mainly to the antiadhesion efficacy. While either injectable hydrogels or RGD modification via chemical binding to enhance cell adhesion have been much investigated, this article distinguishes itself as revealing that a simple mixing of free RGD molecules and a polymeric sol led to an improved injectable system, which could prevent postoperative tissue adhesions very potently.

CONCLUSIONS

A well known cell-adhesive peptide cyclo(RGDfK-) was encapsulated in a thermosensitive physical hydrogel PCLA–PEG–PCLA for prevention of postoperative intestinal adhesions in rabbits. A sustainable release of water-soluble RGD peptides from the physical hydrogel was successfully achieved for 1 week, and this period fits the therapeutic window of anti-adhesion quite well. In vivo experiments confirmed a better efficacy of using RGD-encapsulated hydrogels to prevent tissue adhesions in rabbits than using RGD or hydrogel alone. The operation of this peptide-loaded hydrogel was convenient, and gelation spontaneously occurred at body temperature free of any chemical reaction. Hence, this study affords a facile and efficient system of anti-adhesion biomaterials integrating both pharmaceutical treatments and barrier-based devices. Meanwhile, we have shed new insight into cell-biomaterial interactions by strengthening that cell-adhesive agents could sometimes be used to assist antiadhesion both in vitro and in vivo if the agents are free instead of being immobilized to a substrate.

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