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Surgical suture assembled with polymeric drug-delivery sheet for sustained, local pain relief

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ABSTRACT

Surgical suture is a strand of biocompatible material designed for wound closure, and therefore can be a medical device potentially suitable for local drug delivery to treat pain at the surgical site. However, the preparation methods previously introduced for drug-delivery sutures adversely influenced the mechanical strength of the suture itself – strength that is essential for successful wound closure. Thus, it is not easy to control drug delivery with sutures, and the drug-delivery surgical sutures available for clinical use are now limited to anti-infection roles. Here, we demonstrate a surgical suture enabled to provide controlled delivery of a pain-relief drug and, more importantly, we demonstrate how it can be fabricated to maintain the mechanical strength of the suture itself. For this purpose, we separately prepare a drug-delivery sheet composed of a biocompatible polymer and a pain-relief drug, which is then physically assembled with a type of surgical suture that is already in clinical use. In this way, the drug release profiles can be tailored for the period of therapeutic need by modifying only the drug-loaded polymer sheet without adversely influencing the mechanical strength of the suture. The drug-delivery sutures in this work can effectively relieve the pain at the surgical site in a sustained manner during the period of wound healing, while showing biocompatibility and mechanical properties comparable to those of the original surgical suture in clinical use.

1. Introduction

Post-operative pain originating from the wound is an inevitable inconvenience for patients after surgery. To treat the pain, a pain-relief drug is often administered via the oral route or injection; this approach, however, is limited because of low drug bioavailability at the site of action, as well as unnecessary systemic exposure to the drug [1]. The strategy of local drug delivery can resolve this to a large extent [2], and in this sense a surgical suture – a strand of biocompatible material designed for wound closure – can potentially be used as a suitable medical device for treatment of pain by drug delivery to the local site of surgery.

Previously, surgical sutures have been processed in several different ways for drug delivery: the drug being coated on the suture surface by solution dipping [3,4] or grafting [5], or being encased in the suture thread itself [6]. However, such fabrication procedures can be damaging to the suture and its component materials, adversely influencing the mechanical strength of the suture, which needs to be retained for the purpose of wound closure. For example, when a suture of a poly(*p*-dioxaonone) monofilament was dip-coated with the required drug load, the breaking strain was reported to decrease by more than 30% [4]. Thus, it is not easy to control drug delivery, and the drug-delivery surgical sutures available in clinical use currently are limited to anti-infection purposes [7].

In this work, for the first time to our knowledge, we demonstrate the sutures enabled with controlled delivery of a pain-relief drug and evaluate their in vivo efficacy and biocompatibility. More

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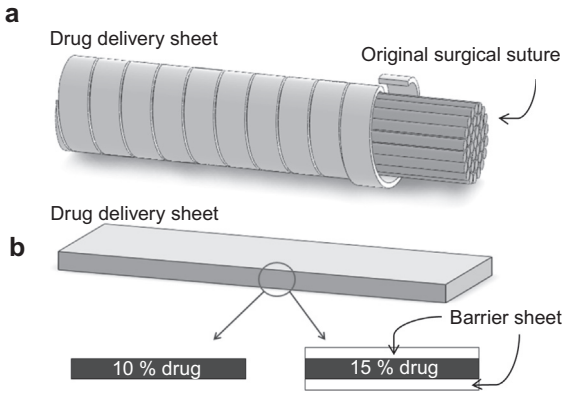

Fig. 1. Schematic procedure for preparation of the drug-delivery sutures. (a) The drug-delivery sheet composed of PLGA and ibuprofen was separately prepared; it was braided and physically attached on top of the surgical suture in clinical use. (b) Two different types of the drug-delivery sheets were prepared for controlled drug delivery: a single-layered sheet (PLGA_IB_S) of PLGA loaded with 10% w/w ibuprofen and multi-layered sheets (M_PLGA_IB_S), where a sheet of PLGA loaded with 15% w/w ibuprofen was sandwiched between the sheets of PLGA only. The single-layered sheet of PLGA without the drug (PLGA_S) was also prepared for comparison.

importantly, we report the fabrication method herein in an effort to maintain the mechanical strength of the original surgical suture. Thus, a sheet of poly(lactic-co-glycolic acid) (PLGA) loaded with a pain-relief drug, ibuprofen, was separately prepared as the delivery carrier (i.e. as a drug-delivery sheet), which was then braided around a surgical suture that had already been approved for clinical use (VICRYL* W9114, Ethicon, USA) (Fig. 1a). The assembled sutures were cured at a temperature slightly above the glass transition temperature (T_g) of PLGA (41–47 °C) but much below the decomposition temperature of ibuprofen (180–300 °C) [8], for better attachment of the sheet around the suture. PLGA is known to be highly biocompatible and biodegradable [9], and it is already one of the major materials forming the biodegradable surgical suture [10]. Ibuprofen, a non-steroidal anti-inflammatory drug, reduces activity of cyclooxygenase producing prostaglandins, which contribute to hyperalgesia at the wounded site [11]. The drug employed for pain relief in this work is already approved by the US Food and Drug Administration for administration via the oral route or injection [12].

We show that, with this physical assembly, the drug release profiles can be tailored for the period of therapeutic need simply by modifying the drug-loaded polymer sheet while the original surgical suture remains intact. With the ability to control delivery, as well as the retained mechanical strength, the drug-delivery suture prepared in this work is shown to effectively relieve the pain in live animals during the period of wound healing.

2. Materials and methods

2.1. Materials

PLGA (50:50; inherent viscosity = 0.41 dl g^{-1}; average MW = 58 kDa) was purchased from Lakeshore Biomaterials (Birmingham, USA). Ibuprofen (assay value ⩾98%) was obtained from Sigma (ME, USA). The surgical sutures in clinical use (VICRYL* W9114) were acquired from Ethicon (USA). Dichloromethane (DCM) and acetonitrile (ACN) were supplied from JT Baker (NJ, USA). Tetrahydrofuran (THF), dimethylformamide (DMF) and o-phosphoric acid were purchased from Daejung (Siheung, Korea), Mallinckrodt (ME, USA) and Sigma-Aldrich (ME, USA), respectively. Zoletil 50 and Rompun were obtained from Bayer (Korea). Paraformaldehyde (4%) was supplied by Dreamcell (Korea).

2.2. Preparation of drug-delivery sutures

We first prepared the drug-delivery sheets by electrospinning (Nano NC, Siheung, Korea) [13]. PLGA was dissolved in a mixed solvent of DCM, THF and DMF to prepare a 30% w/v PLGA solution, where 0, 10 and 15% w/w ibuprofen was dissolved to give a sheet of PLGA only (PLGA_S), a sheet of PLGA and ibuprofen (PLGA_IB_S) and a multi-layered one of PLGA and ibuprofen (M_PLGA_IB_S), respectively. To prepare the PLGA_IB_S, a PLGA and drug solution (10% w/w drug) was electrospun for 100 min. The M_PLGA_IB_S was prepared by electrospinning a PLGA solution without the drug for 50 min, a PLGA and drug solution (15% w/w drug) for 100 min and a PLGA solution without the drug for 50 min. In this way, the M_PLGA_IB_S was composed of the PLGA sheet loaded with the drug in the middle, which was sandwiched between the sheets of PLGA only (Fig. 1b). The PLGA_S was prepared by electrospinning a PLGA solution without the drug for 100 min. The following conditions were used for all electrospinning procedures: applied voltage: 15 kV; tip-to-collector distance: 10 cm; and flow rate: 0.6 ml h^{-1}. Each sheet was then each cut by a hand-held scalpel to give a strand, 1.5 ± 0.5 mm in width, which was then braided around the surface of the surgical suture, as described in Fig. 1a. In this laboratory-scale study, the braiding was conducted carefully by hand under an optical microscope to minimize the overlap or gap between the braided sheets (Fig. S.1). The sheets were used as obtained from the electrospinning, without further treatment, where their mechanical properties were acceptable for braiding. The sutures covered with the sheet were incubated at 47 °C for 1 h while the suture and sheet were slightly strained.

2.3. Characterizations

The sutures and the sheets (i.e. PLGA_S, PLGA_IB_S and M_PLGA_IB_S) prepared in this work were imaged by scanning electron microscopy (SEM; 7501F, Jeol, Japan). The X-ray diffraction (XRD) patterns of the sheets were examined by an X-ray diffractometer (D/MAX RINT 220-Ultima, Rigaku, Japan) equipped with Ni-filtered Cu K_α radiation (λ = 1.5418 Å), where the samples located on a glass substrate were each continuously scanned in a constant rate (2° min^{-1}) at a tube voltage of 40 kV and a current of 30 mA [14]. Thermal properties of the sheets were investigated by differential scanning calorimetry (DSC2901, TA instruments, DE, USA). The samples were each placed in a hermetic pan under nitrogen gas flow, where the temperature was raised from 20 °C to 100 °C at 3 °C min^{-1}.

2.4. Mechanical tests of sutures

Tensile strength of the sutures (i.e. original, PLGA_S, PLGA_IB_S and M_PLGA_IB_S sutures) was examined by the straight-pull and knot-pull tests, using a universal testing machine (UTM; Instron-5543, MA, USA) equipped with a load cell of 1 kN [15]. The samples having a gauge length of 150 mm were pulled at a cross-head speed of 200 mm min^{-1}. During the test, the sample extension and applied load were recorded and calculated to tensile strain and strength, respectively.

2.5. In vitro drug release study

The drug-delivery sutures were each cut to a strand, 4 cm in length, and were immersed in 2 ml of phosphate-buffered saline (PBS; pH 7.4) at 37 °C with continuous agitation. At scheduled intervals, 1 ml of the aliquot was withdrawn and replaced with 1 ml of fresh PBS. The collected aliquot was measured spectrophotometrically (UV-1800, Shimadzu, Japan) at a wavelength of 264 nm.

2.6. Preparation of animal models

We prepared the pain-induced animal models using male Sprague–Dawley rats at an age of 8 weeks and weight of 250–300 g. The protocol was approved by the Institutional Animal Care and Use Committee at Seoul National University Bundang Hospital (BA1107-086/041-01). To induce the pain, an incision, 1 cm in length, was made on the quadriceps femoris muscle of the right leg of the rat under anesthesia, where a 0.1 ml kg^{-1} cocktail of Zoletil 50 and Rompun (1:1 v/v) was injected intraperitnoneally into each rat. The pain-induced animals were then assigned to the four different groups, where the muscles were sutured with the original, PLGA_S, PLGA_IB_S and M_PLGA_IB_S sutures, all 4 cm in length, respectively, and the skin incision was closed with a nylon suture (4–0 nylon suture, Ethicon, USA) for all animal groups. The sham group had an incision on the skin only, closed with a nylon suture (i.e. no incision on the muscle).

2.7. In vivo pain evaluation

The motility of the animal groups was examined to evaluate the pain-relief efficacy of the drug-delivery sutures. For each of the groups, at least five animals were tested for statistics. The animals were examined between 9 a.m. and 1 p.m. in a room on a normal light/dark cycle during the period of 13 days after surgery. The motility of the untreated, normal rats was also recorded to provide the data from the naïve group. To assess the rearing activity, the rats were each placed in a clean, clear vivarium plastic cage (31 cm × 31 cm × 19 cm), where 16 pairs of infrared beams were set 12 cm above the ground (photo-beam and video motion analysis, In Electronics Design, Korea). During 15 min, the rearing activities were each counted at the time when an infrared beam was disrupted. For the gait analyses, the animals were each allowed to walk through a transparent plastic cage (150 cm × 13 cm × 16 cm) while the bottom of the cage was recorded by a video camera (HVR3300CA, High Vision, Korea) at 11–50 frames s^{-1} [16]. The videos were then analyzed to assess the following gait parameters: velocity, stride length, stride duration, stance duration and double stance duration. All rats tested in this work stayed alive until the end of the experiment without showing any noticeable complications after surgery ($n = 35$). The gait analyses performed in this work are described more in detail in the Supplementary Information.

The pain-induced animals were also treated with conventional oral administration of the drug and compared with the other animal groups treated with the sutures. A dose of 25 mg kg^{-1} ibuprofen suspended in 1 ml normal saline was intragastrically administered to the pain-induced rats, where the muscle and skin were sutured with the original and nylon sutures, respectively, 24 h after surgery. The motion analyses described above were performed 30 min after oral administration of the drug, when the highest systemic exposure of the drug is expected (i.e. T_{max} of orally administered ibuprofen) [17].

2.8. Histological examination

To examine biocompatibility of the sutures, the animals were euthanized at scheduled intervals after surgery and the tissue around the suture was obtained. The resulting tissue was then gently rinsed in saline and fixed in paraformaldehyde (4%), which was then microtomed to give the 5 μm thick sections. The sections were each stained with hematoxylin–eosin and imaged using a

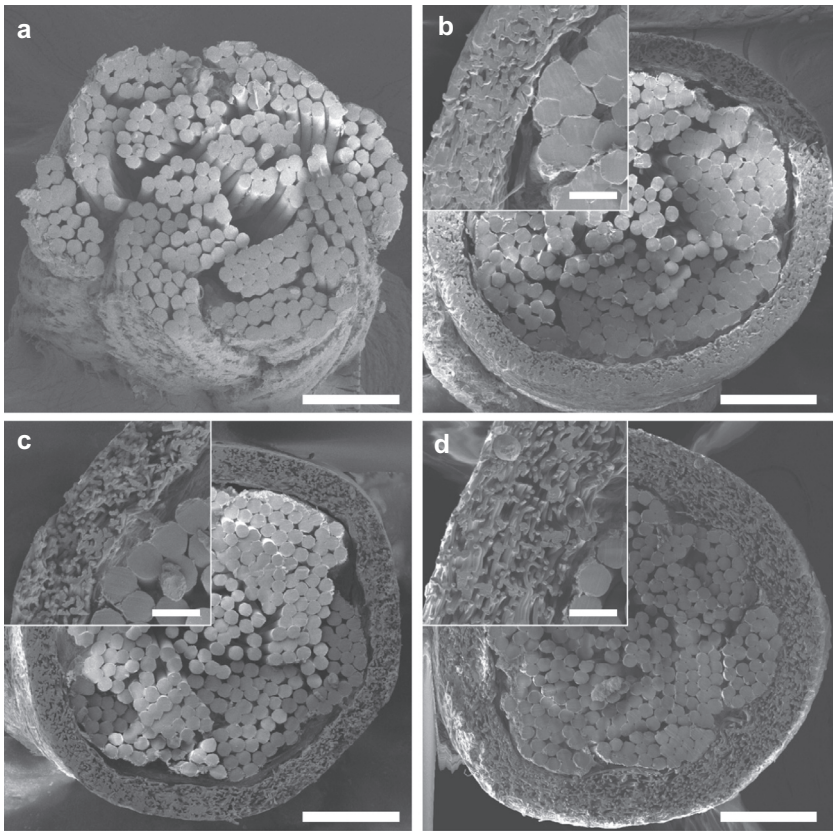

Fig. 2. SEM cross-sectional images of (a) original suture, (b) PLGA_S suture, (c) PLGA_IB_S suture and (d) M_PLGA_IB_S suture. The scale bars are 100 μm in the main images and 10 μm in the insets.

light microscope (X4; Carl Zeiss, Germany), where the inflammatory reaction was analyzed by a professional pathologist. The extent of inflammation was quantitatively assessed by measuring the length between the boundary of the suture thread and end point of neovascularization area around the suture [18]. For each of the tissue samples, we measured eight different end points angularly displaced by 45° around the suture. At each time point and at each group, at least five animals were tested for statistics.

2.9. Statistics

To assess the rearing activities of the 9 sampled days after pain-inducing surgery (i.e. days 1, 2, 3, 4, 6, 7, 9, 11 and 13), the comparisons were made between the sham and the other animal groups by means of the Kruskal–Wallis test, followed by the Bonferonni correction ($p < 0.005$) [19]. The same assessment was performed to analyze the rearing activity and gait velocity only on the first day after surgery, including the data obtained with the oral drug administration group ($p < 0.05$). For gait analysis during the whole tested period after surgery, the generalized estimating equation (GEE) method was applied to evaluate the certain motility parameter of each group with the contrast analysis by calculating the predicted value at each time point. Since the repeatedly measured parameters (gait velocity, stride length, stance duration, stride duration, swing duration and double stance duration) of a subject are not independent of each other, a correction must be made for these within-subject correlations. With the GEE, this correction is carried out by adding a correlation structure as a covariate to the analysis [20,21]. The sham group was set as the reference group in the GEE models and post hoc analyses were conducted to determine significant differences in outcome measures between groups. p-values of less than 0.05 were considered to be statistically significant.

3. Results and discussion

3.1. Characteristics of drug-delivery sheets and sutures

We studied the drug release profiles with two different types of drug delivery sheets prepared in this work: single-layered and multi-layered drug-delivery sheets (i.e. PLGA_IB_S and M_PLGA_IB_S, respectively) (Fig. 1b). The PLGA_IB_S was composed of PLGA with 10% w/w drug and the M_PLGA_IB_S was made by sandwiching a PLGA sheet containing 15% w/w drug between the sheets of PLGA only. The sheets of PLGA only would serve as additional diffusion barriers, thereby prolonging drug release. The single-layered sheet of PLGA only (PLGA_S), i.e. the sheet without the drug, was also prepared for comparison. Thus, three different types of sutures were prepared in this work to give the PLGA_S, PLGA_IB_S and M_PLGA_IB_S sutures with total sheet thicknesses of 29.3 ± 0.7 μm, 28.1 ± 0.5 μm and 51.3 ± 1.5 μm, respectively. Fig. 2 shows scanning electron micrographs of the cross-sections of the sutures prepared in this work. The original surgical suture used in this work was composed of multiple filaments and the diameter of the suture itself was 338.4 ± 34.8 μm (Fig. 2a). After attaching the sheets, the diameters increased to 394.3 ± 13.8 μm, 400.7 ± 11.4 μm and 411.1 ± 4.3 μm for the PLGA_S, PLGA_IB_S and M_PLGA_IB_S sutures, respectively, all showing the physically assembled sheets surrounding the original suture (Fig. 2b–d). The sheets exhibited a nanofibrous structure, as prepared by electrospinning (Fig. S.1). The drug loading amounts were highly reproducible: 32.70 ± 2.30 μg cm^{-1} and 48.27 ± 1.35 μg cm^{-1} for

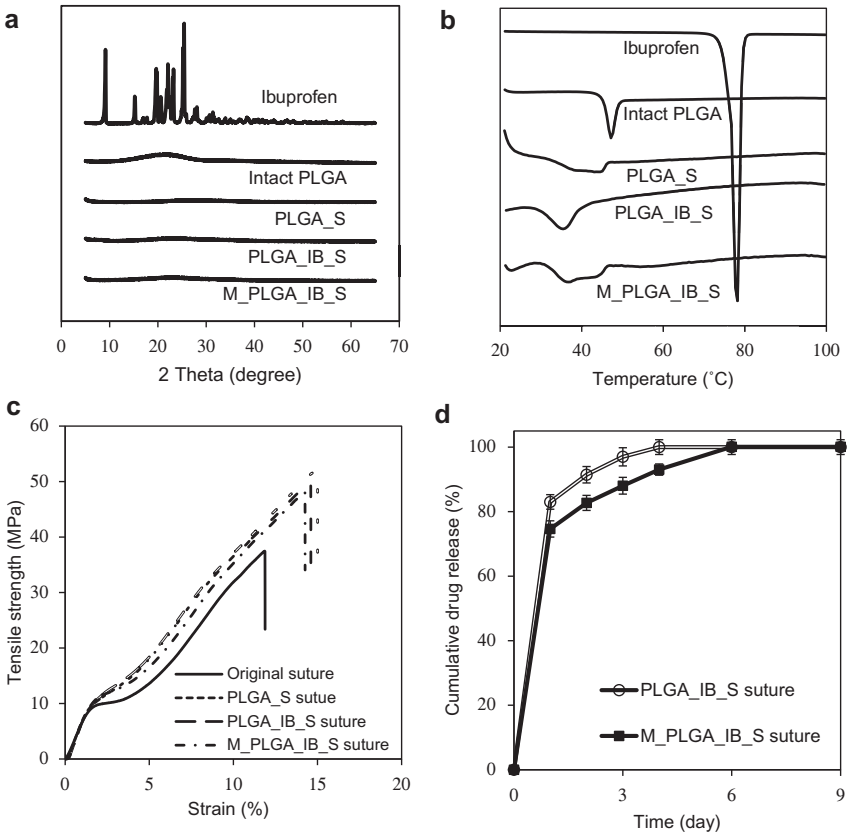

Fig. 3. Characterization of the drug-delivery sheets with the (a) X-ray diffraction and (b) differential scanning calorimetry analyses. (c) Stress–strain curves from the straight-pull test of the sutures. (d) In vitro drug release profiles with the PLGA_IB_S and M_PLGA_IB_S sutures.

Table 1
Mechanical properties of the sutures examined by straight-pull and knot-pull tests.

Test type	Suture type	Tensile strain at break (%)	Tensile strength at break (MPa)
Straight-pull test	Original suture	11.62 ± 1.58	36.69 ± 5.47
	PLGA_S suture	13.87 ± 0.94	49.71 ± 2.36
	PLGA_IB_S suture	14.64 ± 0.76	51.20 ± 1.82
	M_PLGA_IB_S suture	13.75 ± 1.20	48.84 ± 3.07
Knot-pull test	Original suture	24.23 ± 0.59	31.70 ± 0.63
	PLGA_S suture	26.05 ± 4.82	34.91 ± 0.30
	PLGA_IB_S suture	30.28 ± 1.16	35.58 ± 2.28
	M_PLGA_IB_S suture	30.19 ± 2.15	35.47 ± 4.30

PLGA_IB_S and M_PLGA_IB_S sutures, respectively. This result would suggest that the cutting and braiding procedure performed in this work is also reproducible.

We obtained the XRD patterns as shown in Fig. 3a. The characteristic peaks of intact ibuprofen were not observed with the drug-delivery sheets, implying that the drug molecules be distributed in the polymer chains without forming crystalline aggregates [22]. The results from differential scanning calorimetry (DSC) further confirmed this amorphous distribution of ibuprofen in the sheets, as shown in Fig. 3b. An evident endothermic peak at 78.0 °C was observed with intact ibuprofen due to melting of drug crystalline, which, however, was not seen with the PLGA_IB_S and M_PLGA_IB_S. The thermal treatment employed in this work for sheet attachment around the suture did not appear to influence this amorphous distribution profile of the drug in the sheet (Figs. S.2 and S.3). The PLGA_S exhibited a (T_g at ~41.6 °C, which was shifted to a lower temperature, as compared with intact PLGA, due to a large surface area and entrapped air in the nanofibrous structure of the sheet [23]. The T_g was lowered further to 35.5 °C with the PLGA_IB_S as the drug molecules had the polymer chains move easily. The DSC curve with the M_PLGA_IB_S exhibited two distinct glass transitions at ~36.8 °C and ~41.3 °C, which could be ascribed to the drug-loaded PLGA sheet at the core and the surrounding sheets of PLGA only, respectively. For all drug-delivery sheets in this work, both PLGA and ibuprofen were seen to be present without a major change in their chemical structure (Fig. S.4).

To examine the mechanical property of the suture after sheet attachment, we performed the straight-pull and knot-pull tests, already well-established tests for the evaluation of surgical sutures [24]. According to the straight-pull test, the ultimate tensile strength (UTS) increased by 35%, 39% and 33% for the PLGA_S, PLGA_IB_S and M_PLGA_IB_S sutures, respectively, as compared with the original suture (Fig. 3c, Table 1). The knot-pull test also exhibited improved UTS for the sutures with sheet attachment, where the UTS increased by 10%, 12% and 12% for PLGA_S, PLGA_IB_S and M_PLGA_IB_S sutures, respectively (Table 1). These results implied that the preparation method employed in this work did not degenerate the mechanical property of the original surgical

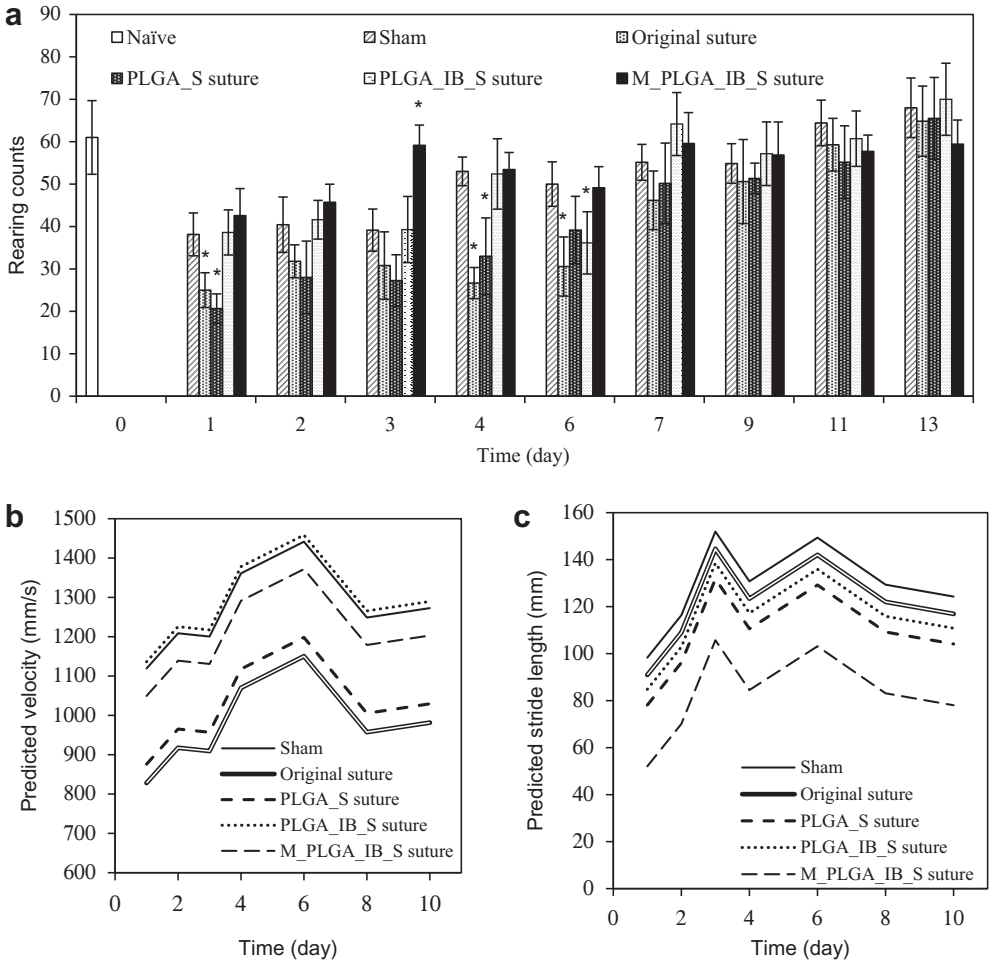

Fig. 4. Results from in vivo pain evaluation. (a) In vivo rearing activity tested during the first 13 days after pain-inducing surgery. *Statistically significant difference compared with the sham ($p < 0.005$). The predicted values of (b) velocity and (c) stride length from the gait analyses were obtained from the statistic GEE model [18,19].

suture but rather improved it due to the addition of the polymeric sheets.

The in vitro drug release experiments showed that the drug could be released in a sustained manner with the drug-delivery sutures (Fig. 3d). Notably, drug release from the M_PLGA_IB_S suture could be more sustained due to the presence of an additional diffusion barrier (i.e. the sheets of PLGA only covering the drug-loaded sheet of PLGA at the core). Thus, the period of drug release could be modulated for up to 4 days and 6 days with the PLGA_IB_S and M_PLGA_IB_S sutures, respectively. The amounts of drug loading were 32.70 ± 2.30 μg cm^{-1} and 48.27 ± 1.35 μg cm^{-1} for PLGA_IB_S and M_PLGA_IB_S sutures, respectively, hence a greater amount of released drug with the M_PLGA_IB_S suture. The rationale determining the drug loading amount in the suture is described in the following. The daily oral dose of ibuprofen for effective pain relief to the rat is known to be 100 mg kg^{-1} [25], hence a maximum dose of 30 mg for a 250–300 g rat was used used in this work. Assuming the complete absorption and homogeneous distribution of the drug in the rat body, the drug available in the quadriceps femoris muscle would be 600 μg since the weight of this muscle is known to be ~6 g per 300 g rat [26]. As we wanted to deliver much less drug locally at the site of surgery, a 20-times less daily dose was suggested in this work: 30 μg drug per day. To deliver this amount of drug for 4 days and 6 days, the total doses would be 120 μg and 180 μg, respectively, and thus the theoretical loading amounts of the drug in the suture should be 30 μg cm^{-1} and 45 μg cm^{-1}, considering the 4 cm length of the suture used for wound closure in the muscle. In this work, the drug loading amounts of the PLGA_IB_S and M_PLGA_IB_S sutures were determined, based on those theoretical values, thereby again giving the actual loading amounts of 32.70 ± 2.30 μg cm^{-1} and 48.27 ± 1.35 μg cm^{-1}, respectively.

3.2. Pain-relief efficacy of drug-delivery sutures

We examined the in vivo pain-relief efficacy of the sutures, using a pain-induced animal model. For this purpose, an incision was made on the quadriceps femoris muscle of the rats, which was then closed with the different types of suture prepared in this work. The motility of the pain-induced animals was evaluated and compared with that of the sham (i.e. the rat with the skin incision only) to determine the degree of pain: the lower the animal motility, the more the pain. First, we examined the activity of rearing with the animal groups treated with the distinct types of the sutures (Fig. 4a) [27,28]. As compared with the sham, the groups treated with the original and PLGA_S sutures (i.e. the sutures without the drug) exhibited fairly low rearing activity for the first 6 days after surgery. The average rearing counts were statistically significantly lower on days 1 and 4, and days 1, 4 and 6 for the original and PLGA_S sutures, respectively ($p < 0.005$), suggesting the presence of untreated pain on the muscle before wound healing.

On the other hand, improvement in rearing was evident with the groups treated with the drug-delivery sutures. Both PLGA_IB_S and M_PLGA_IB_S suture groups exhibited rearing activity similar to that of the sham during the whole tested period of 9 days. However, there was a noticeable decrease in rearing activity on day 6 with the PLGA_IB_S suture probably due to early completion of drug release. Drug release from the PLGA_IB_S suture was completed in 4 days according to the in vitro drug release experiment (Fig. 3d). Interestingly, for the M_PLGA_IB_S suture group, the rearing activity was significantly higher than that of the sham on day 3. For the first 3 days, a higher amount of drug was released from the M_PLGA_IB_S suture than from the PLGA_IB_S suture: the average cumulative amounts of drug released from the M_PLGA_IB_S and PLGA_IB_S sutures were 169.6 ± 11.4 μg and 126.9 ± 11.3 μg, respectively. This larger amount of drug release might have reduced the pain not only in the muscle but also in the skin with

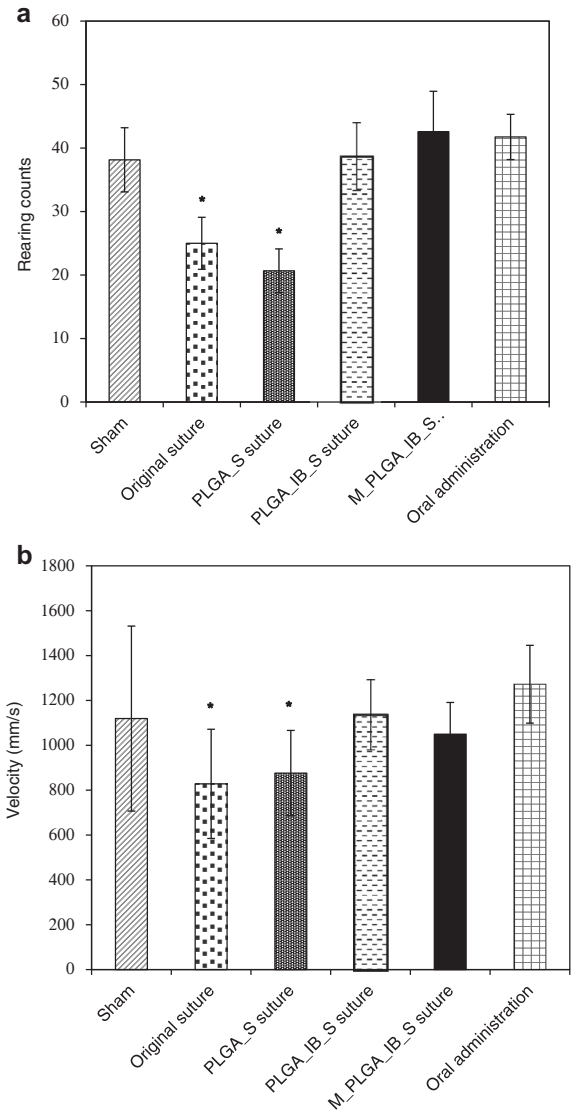

Fig. 5. In vivo pain evaluation on the first day after surgery, where (a) rearing activities and (b) gait velocities were compared, including the results from the animal groups treated with oral administration of ibuprofen. ∗Statistically significant difference compared with the sham ($p < 0.05$).

the M_PLGA_IB_S suture. From day 7, the rearing activity for all groups became not very different and even became similar to that of the naïve group, implying complete wound healing in the muscle afterwards [29]. It should be noted that a sustained pain-relief efficacy could be obtained with a biphasic drug-release profile of the sutures: more than 70% of the drug was released in 1 day and the rest for another 3–5 days, with a low release rate of 6–8% day^{-1} (Fig. 3d). It appeared that a large amount of drug release effectively relieved the severe pain right after surgery, and afterwards the pain could be reduced even with a slight exposure to the drug. For this reason, the pain was often treated with step-down regimen in clinical practice, starting from a higher to lower drug dose [30]. As the effect of ibuprofen was reported to be dose-dependent [31], a lower drug-dose on day 1 may not effectively relieve the severe pain right after surgery in this work.

To further confirm the pain-relief efficacy, we also performed the gait analyses with the pain-induced animal groups [32,33]. Among all gait parameters tested in this work (Fig. S.5), the predicted gait velocity and stride length provided statistically meaningful data (Fig. 4b and c). The predicted velocity with the drug-delivery sutures (i.e. the PLGA_IB_S and M_PLGA_IB_S sutures)

was similar to that of the sham (Video S.1) while the decrease in velocity was statistically significant with the original suture group ($p < 0.05$), suggesting effectively reduced pain in the muscle with the drug-delivery sutures. Notably, among all groups, a dramatic decrease in predicted stride length was observed with the M_PLGA_IB_S suture (Fig. 4c). This result implied even less pain with the M_PLGA_IB_S suture group than with the sham ($p \approx 0.05$), which again could be attributed to a higher amount of drug release (Fig. 3d), thereby relieving the pain even in the skin.

We also sought to compare the pain-relief efficacy of the drug-delivery sutures with that of conventional oral drug therapy. As shown in Fig. 5, both rearing activity and gait velocity of the oral administration group were similar to those of the sham while both parameters were statistically significantly lower with the original and PLGA_S suture groups ($p < 0.05$). This indicated that orally administered ibuprofen could effectively relieve the pain with the dose employed in this work, which is in a ranging dose commonly recommended for use in rodents and rabbits and about a half of the dose previously used to effectively reverse the pressure pain on the sensitized hind paw of the rats [17,34]. It should be noted that this similar efficacy of pain relief could be achieved with a much lower dose of ibuprofen from the drug-delivery suture prepared in this work. For example, we used the PLGA_IB_S and M_PLGA_IB_S sutures, each 4 cm in length, for wound closure on the muscle, giving ~100 μg and 140 μg ibuprofen released during the first day (Fig. 3d), respectively. On the other hand, considering the oral regimen of ibuprofen for continuous pain relief, often four dosing times per day, the daily oral dose should be represented as 100 mg kg^{-1}, i.e. at least 2500 μg per rat, which, therefore, is 25 and 18 times larger than those of the PLGA_IB_S and M_PLGA_IB_S sutures, respectively.

3.3. Biocompatibility of drug-delivery sutures

To examine biocompatibility, we performed histological analyses on the tissues around the sutures prepared in this work. A classic grading method to categorize inflammation would not be reliable in this work due to unregulated dispersion of inflammation

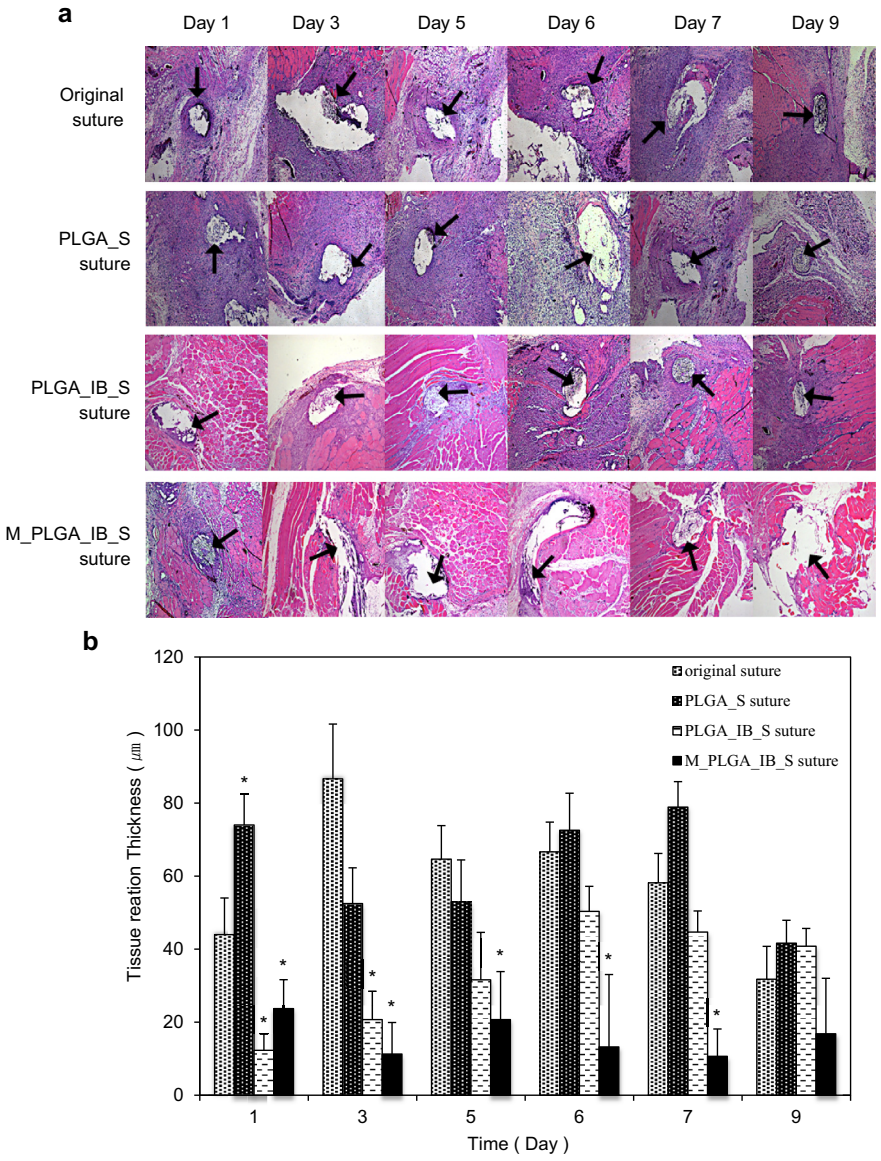

Fig. 6. Histological analyses of the tissue in the quadriceps around the suture during the period of drug release. (a) Histological images and (b) degree of inflammation obtained around the suture at timed intervals after pain-inducing surgery. The arrows in (a) show the locations of the sutures within the tissue. *Statistically significant difference compared with the original suture ($p < 0.05$).

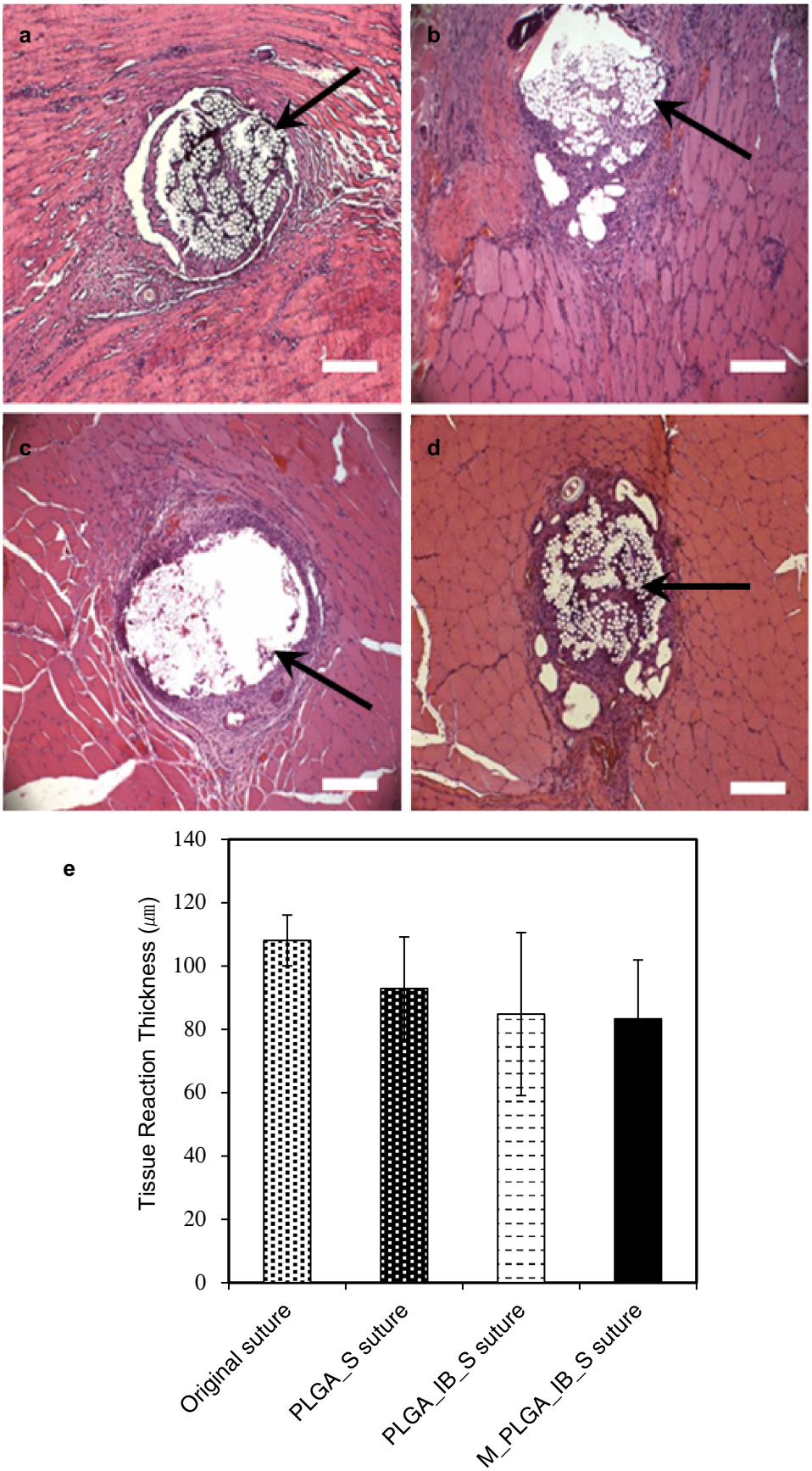

Fig. 7. Histological analyses of the tissue in the quadriceps around the suture 14 days after surgery. The histological images from (a) original suture, (b) PLGA_S suture, (c) PLGA_IB_S suture and (d) M_PLGA_IB_S suture, where the arrows indicate the locations of the suture in the tissue. The scale bars are 100 μm. (e) Quantitative evaluation of tissue inflammation reaction, measuring the length between the boundary of the suture thread and end point of neovascularization area around the suture [16].

cells around the suture. Therefore, we measured the thickness of the inflammatory tissue reaction instead, following the protocol from the previous study (Fig. S.6) [18]. During 7 days after surgery on the muscle (i.e. during the period of active wound healing), the PLGA_IB_S and M_PLGA_IB_S suture groups exhibited fairly low inflammation, as compared with the original and PLGA_S suture ones. As shown in Fig. 6, there was a marked decrease in cellularity of inflammatory cells around the PLGA_IB_S and M_PLGA_IB_S suture groups until day 5, compared to those of the groups treated with the sutures without the drug (i.e. the original and PLGA_S suture groups). This result implied sustained, local exposure of ibuprofen, an anti-inflammatory drug, around the PLGA_IB_S and M_PLGA_IB_S sutures. However, from day 6, a rebound inflammatory reaction was seen with the PLGA_IB_S suture group possibly due to early completion of ibuprofen release. On the other hand, the M_PLGA_IB_S suture group exhibited a low degree of inflammation at all times tested in this work. After day 9, following muscle recovery, there was no significant difference in inflammation among all groups.

A fairly long time after drug release was already completed (day 14), the tissue reaction to the PLGA_IB_S and M_PLGA_IB_S sutures was again not very different from that to the PLGA_S and original suture (Fig. 7) [35], exhibiting mild granulomatous inflammation and typical inflammatory reaction with infiltrate of mixed cells, such as foreign body type multinucleated giant cells, fibroblasts, lymphocytes and plasma cells. Among all sutures, the extents of the tissue inflammatory reaction were also not significantly different (Fig. 7e) [18], implying that biocompatibility of the drug-delivery sutures prepared in this work is comparable to that of the original surgical suture in clinical use.

4. Conclusion

To treat local pain after surgery, we suggest surgical suture enabled with drug delivery in this work. With simple, physical assembly of the surgical suture and polymeric sheet loaded with a pain-relief drug, controlled drug release can be achieved by modifying only the sheet for drug delivery, hence maintaining the mechanical strength of the suture suitable for surgical wound closure. In this work, the period of drug release could be varied for up to 6 days with the drug-delivery sutures, which also exhibited the increased UTS as compared with the original surgical suture. We demonstrate that the drug-delivery suture can relieve the pain effectively at the wounded site without compromising biocompatibility of the original surgical suture. In this study, the sutures assembled with the sheets of PLGA for ibuprofen delivery exhibited the apparent pain-relief efficacy, similar to that treated with oral drug administration, even with the lower dose, and fairly good in vivo biocompatibility during the whole period when an acute post-operative pain was expected to be present. Therefore, we conclude that the drug-delivery surgical suture suggested in this work is a novel system for postoperative pain relief. Moreover, we anticipate that the fabrication method introduced in this work will allow the surgical suture to be modifiable for delivery application of numerous different drugs while retaining its mechanical strength.

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Appendix A. Figures with essential colour discrimination

Certain figures in this article, particularly Figs. 1, 3–7, are difficult to interpret in black and white. The full colour images can be found in the on-line version, at http://dx.doi.org/10.1016/j.actbio.2013.06.003.

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actbio.2013.06.003.

References

[1] Rømsing J, Møiniche S, Østergaard D, Dahl J. Local infiltration with NSAIDs for postoperative analgesia: evidence for a peripheral analgesic action. Acta Anaesthesiol Scand 2000;44:672–83.
[2] Gerwin N, Hops C, Lucke A. Intraarticular drug delivery in osteoarthritis. Adv Drug Deliv Rev 2006;58:226–42.
[3] Wang L, Chen D, Sun J. Layer-by-layer deposition of polymeric microgel films on surgical sutures for loading and release of ibuprofen. Langmuir 2009;25:7990–4.
[4] Zurita R, Puiggalí J, Rodríguez-Galán A. Loading and release of ibuprofen in multi and nonofilament surgical sutures. Macromol Biosci 2006;6:767–75.
[5] Gupta B, Jain R, Singh H. Preparation of antimicrobial sutures by preirradiation grafting onto polypropylene monofilament. Polym Adv Technol 2008;19:1698–703.
[6] He CL, Huang ZM, Han XJ. Fabrication of drug-loaded electrospun aligned fibrous threads for suture applications. J Biomed Mater Res, Part A 2009;89:80–95.
[7] Chang WK, Srinivasa S, Morton R, Hill AG. Triclosan-impregnated sutures to decrease surgical site infections: systematic review and meta-analysis of randomized trials. Ann Surg 2012;255:854.
[8] Tita B, Fulias A, Stefanescu M, Marian E, Tita D. Kinetic study of decomposition of ibuprofen under isothermal conditions. Methods 2011;15:29–32.
[9] Acharya S, Sahoo SK. PLGA nanoparticles containing various anticancer agents and tumour delivery by EPR effect. Adv Drug Deliv Rev 2011;63:170–83.
[10] Pillai CKS, Sharma CP. Review paper: absorbable polymeric surgical sutures: chemistry, production, properties, biodegradability, and performance. J Biomater Appl 2010;25:291–366.
[11] Kurumbail RG, Stevens AM, Gierse JK, McDonald JJ, Stegeman RA, Pak JY, et al. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. Nature 1996;384:644–8.
[12] Benzon H, Raja SN, Fishman SM, Liu S, Cohen SP. Essentials of Pain Medicine. Saunders E-book; 2011.
[13] Li D, Xia Y. Electrospinning of nanofibers: reinventing the wheel? Adv Mater 2004;16:1151–70.
[14] Khang G, Rhee JM, Jeong JK, Lee JS, Kim MS, Cho SH, et al. Local drug delivery system using biodegradable polymers. Macromol Res 2003;11:207–23.
[15] Yamagami N, Mori R, Yotsumoto T, Hatanaka H, Takao M, Uchio Y. Biomechanical differences resulting from the combination of suture materials and repair techniques. J Orthop Sci 2006;11:614–9.
[16] Gorska T, Majczyński H, Zmysłowski W. Overground locomotion in intact rats: contact electrode recording. Acta Neurobiol Exp 1998;58:227.
[17] Lichtenberger LM, Romero JJ, de Ruijter WMJ, Behbod F, Darling R, Ashraf AQ, et al. Phosphatidylcholine association increases the anti-inflammatory and analgesic activity of ibuprofen in acute and chronic rodent models of joint inflammation: relationship to alterations in bioavailability and cyclooxygenase-inhibitory potency. J Pharmacol Exp Ther 2001;298:279–87.
[18] Kujala S, Pajala A, Kallioinen M, Pramila A, Tuukkanen J, Ryhänen J. Biocompatibility and strength properties of nitinol shape memory alloy suture in rabbit tendon. Biomaterials 2004;25:353–8.
[19] Foley A, Halbert J, Hewitt T, Crotty M. Does hydrotherapy improve strength and physical function in patients with osteoarthritis – a randomised controlled trial comparing a gym based and a hydrotherapy based strengthening programme. Ann Rheum Dis 2003;62:1162–7.
[20] Ghisletta P, Spini D. An introduction to generalized estimating equations and an application to assess selectivity effects in a longitudinal study on very old individuals. J Educ Behav Stat 2004;29:421–37.
[21] McComsey GA, Yau L. Asymptomatic hyperlactataemia: predictive value, natural history and correlates. Antiviral Ther 2004;9:205–12.
[22] Yu DG, Zhang XF, Shen XX, Brandford-White C, Zhu LM. Ultrafine ibuprofen-loaded polyvinylpyrrolidone fiber mats using electrospinning. Polym Int 2009;58:1010–3.

[23] Ramakrishna S. An introduction to electrospinning and nanofibers. World Scientific Pub. Co. Inc.; 2005.
[24] ASTM. Standard Test Method for tensile properties of yarn by the single-strand method. West Conshohocken, PA: ASTM International; 2002.
[25] Wu R, Lamontagne D, de Champlain J. Antioxidative properties of acetylsalicylic acid on vascular tissues from normotensive and spontaneously hypertensive rats. Circulation 2002;105:387–92.
[26] Dogan T, Kryger Z, Zhang F, Shi DY, Komorowska-Timek E, Lineaweaver WC, et al. Quadriceps femoris muscle flap: largest muscle flap model in the rat. J Reconstr Surg 1999;15:433–8.
[27] Buvanendran A, Kroin JS, Kari MR, Tuman KJ. A new knee surgery model in rats to evaluate functional measures of postoperative pain. Anesth Analg 2008;107:300–8.
[28] Horlings CGC, van Engelen BGM, Allum JHJ, Bloem BR. A weak balance: the contribution of muscle weakness to postural instability and falls. Nat Clin Pract Neurol 2008;4:504–15.
[29] Nikolaou PK, Macdonald BL, Glisson RR, Seaber AV, Garrett WE. Biomechanical and histological evaluation of muscle after controlled strain injury. Am J Sports Med 1987;15:9–14.
[30] Schnitzer TJ. Update on guidelines for the treatment of chronic musculoskeletal pain. Clin Rheumatol 2006;25(Suppl 1):S22–9.
[31] Schou S, Nielsen H, Nattestad A, Hillerup S, Ritzau M, Branebjerg PE, et al. Analgesic dose-response relationship of ibuprofen 50, 100, 200, and 400 mg after surgical removal of third molars: a single-dose, randomized, placebo-controlled, and double-blind study of 304 patients. J Clin Pharmacol 1998;38:447–54.
[32] Vrinten DH, Hamers FFT. CatWalk'automated quantitative gait analysis as a novel method to assess mechanical allodynia in the rat; a comparison with von Frey testing. Pain 2003;102:203–9.
[33] Selph J, Boncek V, Soroko F, Harris T, Cochran F. The pharmacologic evaluation of locomotor activity versus inflammatory parameters in rat adjuvant arthritis. Inflamm Res 1993;39:201–3.
[34] Jenkins WL. Pharmacologic aspects of analgesic drugs in animals: an overview. JAVMA-J Am Vet Med Assoc 1987;191.
[35] Barbolt TA. Chemistry and safety of triclosan, and its use as an antimicrobial coating on Coated VICRYL® plus antibacterial suture (coated polyglactin 910 suture with triclosan). Surg Infect 2002;3:45–53.