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Original Article

Evaluation of pharmacokinetics and safety of a long-term estradiol-releasing stent in rat uterine

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ABSTRACT

Purpose: Intrauterine adhesion (IUA), often leading to gynecological complications including amenorrhea, abdominal pain and infertility, is frequently induced by injuries to the endometrium. Hence it would be of great benefit to take efforts to prevent adhesion after intrauterine operations. Orally administration of 17β-estradiol (E2) is commonly used to promote endometrium regeneration, but is limited by low concentrations at the injured sites. We aim at preparing an E2-releasing uterine stent, which could improve the efficiency of E2 therapy and be utilized for IUA prevention.
Methods: We designed a silicone rubber stent, which could be implanted in the uterine cavity and continuously release E2 in long term. Stents were placed in rodent uterine, and removed at different time points. Remaining E2 in stent was measured by high performance liquid chromatography (HPLC), and organ E2 concentrations were detected by enzyme-linked immuno sorbent assay (ELISA). Endometrium morphology was examined by histological staining of paraffin sections.
Results: Our stent showed a controlled release of E2 in rodent uterine for over 60 days, and significantly increased E2 concentration in serum and in situ uterine. After the stent was removed from uterine, E2 rapidly reverted to a normal level. Also, the stent did not induce pathological changes in endometrium.
Conclusions: The uterine stent provided abundant local E2 in uterine cavity with satisfactory safety. The silicone rubber based E2-releasing uterine stent could be further advanced by adjusting its shape and E2 load for its clinical application, and might promisingly help lowering the incidence of IUA.

1. Introduction

Intrauterine adhesion (IUA) is a consequence of endometrial trauma, which results in partial or complete obstruction of the cervical canal or uterine cavity. IUA is clinically defined as Asherman syndrome, and characterized by symptoms of hypomenorrhea, amenorrhea, infertility, lower abdominal pain or recurrent pregnancy loss [1]. IUA could arise from injuries of endometrial basal layer, due to curettage, cesarean section or hysteromyomectomy. Infections such as tuberculosis could also induce adhesion by causing chronic endometrial inflammation [2]. Disorder in endometrial homeostasis may increase susceptibility of endometrial fibrosis. However, the exact pathogenesis of IUA is vague [3].

IUA is frequently encountered in approximately 20% women after miscarriage, according to a meta-analysis [4]. Hysteroscopic adhesiolysis is the current preferred management for IUA [5,6]. Nevertheless, the spontaneous recurrence rate after invention reached nearly 30% [7]. Moreover, impaired biochemical and vascular environment in the endometrium increases incidence of pregnancy complications [8]. Therefore, it is still necessary to seek for effective strategies to prevent the occurrence of IUA after invasive operations in the uterine cavity.

To date, several approaches have been developed for postoperative prevention. A common choice is to set physical

Abbreviations: IUA, intrauterine adhesion; IUD, intrauterine devices; E2, 17β-estradiol; AUC, area under the curve; PLGA, poly lactic-coglycolic acid; HAECM, human amniotic extracellular matrix; HP, heparin-poloxamer.

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barriers to avoid attachment of injured endometrium. Intrauterine devices (IUDs), Foley's catheter balloon, hydrogels and biofilms are applied for this purpose [9–11]. A second direction is to use 17β-estradiol (E2) or growth factors to promote endometrial regeneration [12,13]. In addition, cell-based therapy, especially stem cell therapy has become a novel scheme to promote endometrial repair [14]. Human amniotic epithelial cells, menstrual blood-derived stromal cells, bone marrow mesenchymal stem cells and human umbilical cord-derived mesenchymal stem cells reveal great potentials in IUA treatment, and the latter two cell types achieved proceeds in clinical trials [15–18]. These strategies are often combined for better efficacy.

Here, we attempt to prepare a silicone rubber stent-based E2 releasing system, aiming at providing E2 continuously within a long period, and offering a structural barrier for endometrium. To provide foundation for further clinical advance, we assessed the safety and pharmacokinetics of this device in female rats. We implanted E2-releasing stents in one side of the rat uterine, and took the stents out at different time points. In this way, we measured the remaining E2 in stents, and E2 concentration in blood and organ supernatants to estimate E2-releasing efficacy and organic residual. We also examined the uterine morphology to evaluate local safety. Our work made preliminary assessment of an E2-releasing stent targeting IUA prevention. With fine local E2 delivery efficiency and safety, this device could be applied for clinical use after further improvement.

2. Material and methods

2.1. Animal models

All animal procedures were approved by the Institutional Animal Care and Use Committee of Shanghai and were performed in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals. Animal experiments were carried out in accordance with National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). 8-week female Sprague Dawley rats, weighing 200–250 g each were purchased from Vital River Laboratory Animal Technology (Zhejiang, China). Rats were maintained in SPF conditions after adapted to the new environment for at least a week.

Animals were randomly divided into 3 groups, including the control group (without intervention), the intragastric group (intragastricly administrated with estradiol valerate) and the stent group (implanted with E2-releasing uterine stent). The latter two groups were further divided into more sub-groups, to collect specimens at different time points of 3 h, 1 d, 3 d, 7 d, 14 d, 28 d, 42 d, 60 d or 90 d after stent implantation or first intragastric administration. In addition, the stent group contained 3 more sub-groups of 120 d, 180 d and 365 d to evaluate long-term impacts after stent extraction. Each group contained at least 6 rats (Table 1).

The control group did not undergo E2 administration. The intragastric group received intragastric administration of estradiol valerate suspension daily at 09:00 am. For the stent group, an operation was performed to implant the estradiol-releasing stent in the right uterine horn. The longest period of estradiol treatment was 60 days. For the sub-groups with a posterior end-point, at the 60th day, the intragastric administration terminated, or the stent was extracted by surgical procedures. For the rats in 90 d, 120 d, 180 d and 365 d sub-groups, they were maintained until the end point (Fig. 1).

Table 1
Allocation of animals.

Time of E2 administration	Stent	Intragastric
3 h	6	6
1 d	6	6
3 d	6	6
7 d	6	6
14 d	6	6
28 d	6	6
42 d	6	6
60 d	6	6
90 d	6	6
120 d	6	0
180 d	6	0
365 d	6	0
Control	6	

2.2. Uterine stent and reagents

The E2-releasing stent was manufactured by Puyi Biotechnology (Shanghai, China). The average weigh of a piece of stent for one rat was 7.559 mg, and the initial E2 content in a piece of stent was 1442 μg on average. Estradiol valerate was acquired from Progynova tablets (Bayer Co.).

2.3. Intrauterine stent implantation and extraction

The surgical management was conducted under sterile conditions. The rats were anesthetized by intraperitoneal injection of pentobarbital sodium. The uterus was exposed by an excision in the low midline abdomen. A small incision was made by a 7-gauge needle at the upper portion of the right uterine horn, and a stent was inserted into the uterine cavity from the incision. The abdominal cavity was closed subsequently (Fig. 2ABC).

To remove the implanted stents, rats were anesthetized. After the uterus was exposed, a new incision was made to remove the stent with a forcep. At the time we removed the stents, all stents stayed in situ in the right uterine. The stent was conserved to measure the residual estradiol. For the sub-groups of less than 60 d, rats were sacrificed for organ specimen collection; for the sub-groups of more than 90 d, the abdominal cavity was closed, and rats were maintained until end-point.

2.4. Intragastric administration of estradiol valerate

The maximum dose of orally taken estradiol for a 60 kg-adult is 4 mg/d. The dosage was switched into the biological equivalent dose of rats according to body surface area, so the rats were administrated with 0.42 mg/kg estradiol daily. Progynova (estradiol valerate tablets) were prepared into suspension for intragastric administration.

2.5. Organ specimen collection and management

Intraventricular blood sampling was applied after anesthetized. Blood samples stood for 30 min at room temperature, and were centrifuged at 3,000 rpm at 4 °C for 15 min to obtain serum. After blood sampling, rats were sacrificed. Hearts, livers, spleens, lungs, kidneys and uteruses were dissected, then surrounding tissue was removed. Uteruses in control group and 365 d sub-group of stent group were weighted and photographed for gross examination. All organs were washed in normal saline solution and sucked dry with normal filters. Left and right uteruses were separated. A small

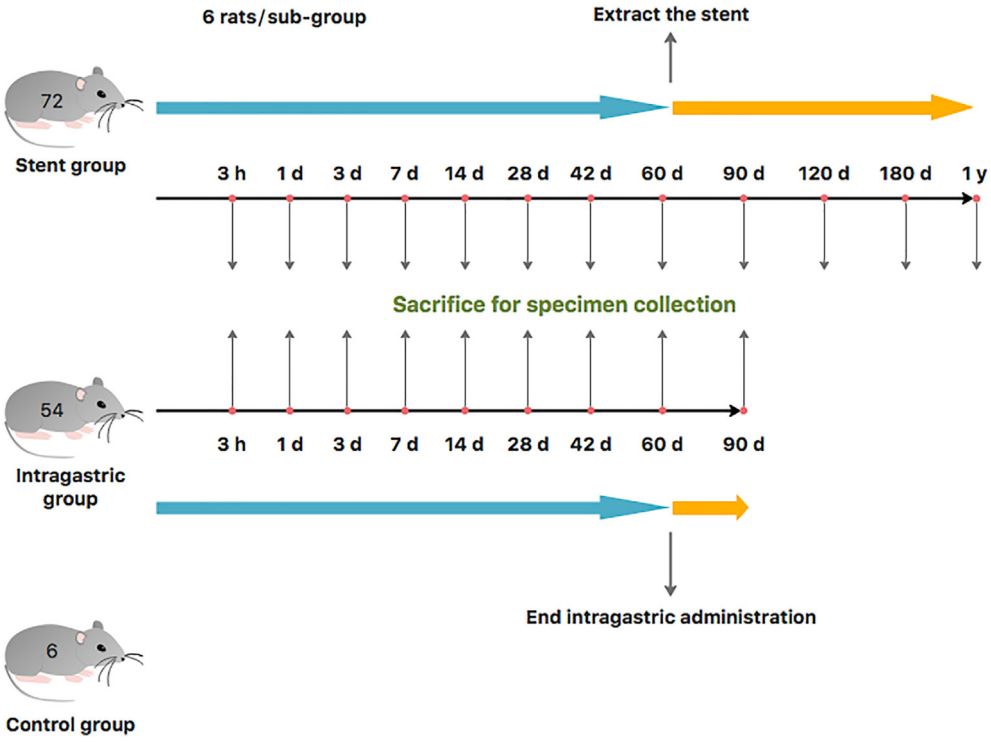

Fig. 1. Design of animal experiments. Female rats were divided into 3 groups. Rats were implanted with E2-releasing stent, intragastricly administrated with estradiol valerate or received no E2 treatment. At each time point indicated, 6 rats exited treatment and were sacrificed for sample collection. Especially, at day 60, surgeries were taken to remove the stents in the remaining implanted rats, and the 90 d sub-group of intragastric group discontinued medication.

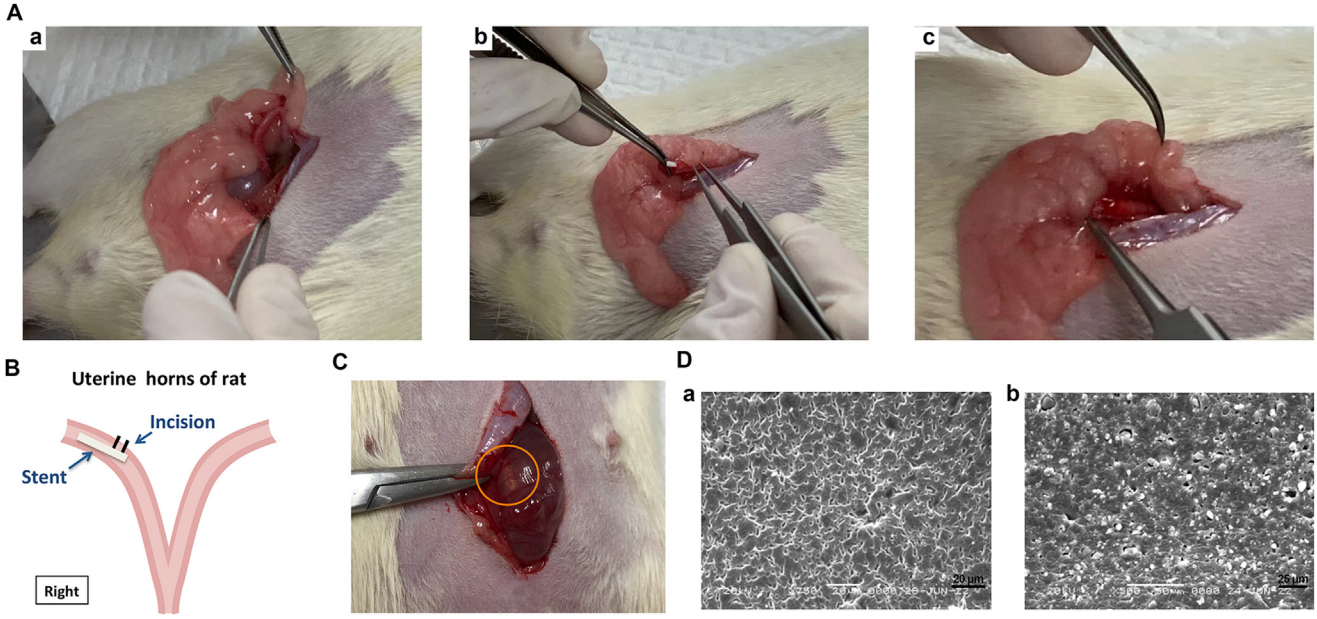

Fig. 2. Implantation and extraction of stents. A. Surgical procedures were taken to implant the stent in rodent uterine. (a) Uterus was exposed by an abdominal incision. (b) The stent was inserted into uterine cavity from a small cut. (c) The entire stent was placed in uterine cavity. B. Stents were placed at the upper portion of the right uterine horn. C. At the time of stent removal, the stent was in place. D. SEM images of silicon rubber stents. (a) Photograph of stent surface. (b) Photograph of stent cross section.

section of organ specimen was weighted, placed in a centrifuge tube, added with 10-fold PBS, and homogenized. The tissue homogenate was centrifuged at 3000 rpm at 4 °C for 15 min to collect the supernatant. Serum and supernatants were stored in -80°C until use.

2.6. Detection of E2 concentration

The levels of rat estradiol in serum and organs were analyzed using a relevant enzyme-linked immuno sorbent assay (ELISA) kit (OSD Biotechnology, Hunan, China) according to the manufacturer's

instructions. The serum was diluted 1:2, and organ supernatants were diluted 1:50 for the ELISA assay.

Remaining E2 in uterine stent was measured by HPLC. After extracted from the uterine, the stent was immersed in trichloromethane at 50 °C for 6 h. The immersed solutions were filtered through a poly tetra fluoroethylene filter, and samples were analyzed by HPLC equipped with a C18 column. E2 concentration was determined using an established calibration curve.

2.7. Morphology analysis

Uterus specimens were collected, fixed in 4% paraformaldehyde overnight, dehydrated in graded alcohols, cleared in xylene and embedded in paraffin. The embedded tissues were sliced into 5-μm-thick sections transversally. Hematoxylin and eosin (H&E) staning was applied to observe the morphological structure of uterine. Masson's trichrome staining was applied to evaluate endometrial stromal fibrosis.

The morphology and structure of stents were observed with scanning electron microscopy (JEOL, JSM-5600LV). The samples were coated by ion sputter gold under vacuum.

2.8. Statistical analysis

Data are expressed as mean ± standard deviation (SD). Student's *t* test or one-way analysis of variance (ANOVA) was performed to analyze the data. Statistics were calculated using GraphPad Prism version 6 (GraphPad software, La Jolla, USA). A p value < 0.05 was considered to be statistically significant.

3. Results

3.1. Silicone rubber uterine stent revealed a slow release effect of E2 in vivo

The basic material of stent was silicone rubber with micropore structure (Fig. 2D). The mechanism of the slow-releasing control could be categorized as matrix device, in this way, E2 was dispersed in the silicone rubber carrier, and could elute out of the stent matrix. Stents were implanted in rodent uterine to study their pharmacological parameters and safety.

To evaluate the characteristics of E2 release from stents in *vivo*, we removed the stents from the uteruses at different time points after intrauterine implantation. The E2 remained in stents were eluted with trichloromethane solvents and measured by HPLC. The released E2 was calculated by the difference of total dose and remaining dose. As shown in Fig. 3 and Supplementary Table S1, from the first 3 h to 60 d after stent install, E2 exhibited a steady-state release profile. E2 release continuously increased until 60 d, the end point of administration. The percent of released estradiol reached 36.5 ± 3.3% at 60 d after implantation. As a whole, the stent was able to serve as a sustained E2-releasing device.

3.2. Serum pharmacokinetics of E2-releasing stent

Rodent E2 plasma concentration was reported to range from 2.4 to 145 pg/mL [19]. We measured the average E2 concentration in serum of the control group (51.2095 pg/mL). This is in accordance with the previous study. As soon as 3 h post implantation, the stent induced a burst of serum E2 concentration, reaching 3728.7 ± 383.51 pg/mL. This is in accordance with the previous report that genital administrated E2 could reach an initial burst in serum with in a few hours [20]. Subsequently, the serum E2 decreased considerably until 28 d post implantation, but still at a supra physiological level of 240 ± 46.872 pg/mL. After 60 d, with the

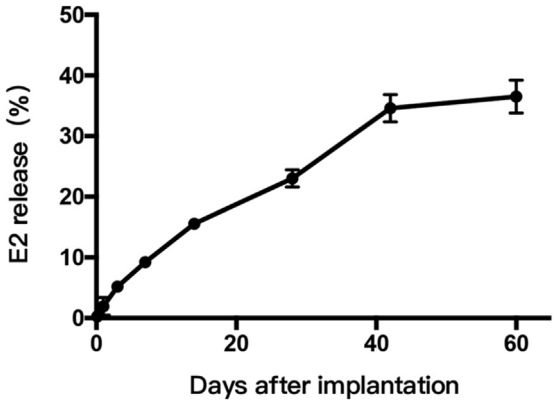

Fig. 3. E2 release in stents *in vivo*. E2 continuously released from silicone rubber during the 60-day period after implantation. E2 releasing rate at each time point is shown in mean and SD.

stent removed from uterine, the serum E2 declined to the physiological range. Afterwards, the serum E2 of stent group maintained within the physiological range, and the concentrations of each subgroup in stent group had no significant difference with the control group (Fig. 4, Supplementary Table S2). From these results, we conclude that the stent has high efficiency in E2 delivery *in vivo*, and provides sustainable serum E2 level within 60 d. Besides, after withdraw of stent E2 administration, the serum E2 rapidly dropped to normal level, indicating that the stent E2 releasing system does not induce E2 accumulation. We calculated the pharmcokinetic parameters of the stent released E2 in serum. The area under the curve (AUC) was 43016 d pg/mL. Cmax was 3729.7 pg/mL. T 1/2 was 1.6 d.

We also measured the serum E2 in intragastric group, to evaluate the differences between stent and oral E2 administration, which is a major clinical route of E2 medication. Serum E2 also reached 99.7 ± 41.116 pg/mL at 3 h in intragastric group, which was a significantly higher level compared with the control group, and progressively increased to a peak of 139.3 ± 72.439 pg/mL at 42 d. However, through out the E2 administration, the serum E2 concentration of oral group was lower than that of stent group. This might be owing to the differences between the bioavailabilities of oral and stent-loaded E2, since orally taken E2 goes through hepatic first-pass effect, while an intrauterine route delivers E2 directly to

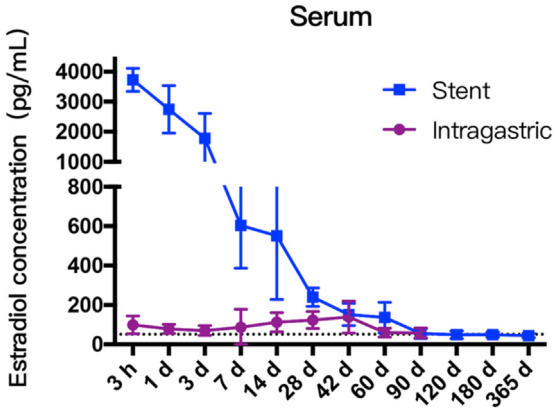

Fig. 4. Serum E2 concentration curve. Plots of cumulative serum concentration of E2 verses time for the stent and intragastric administration are shown as mean ± SD. The dotted line shows the E2 baseline of serum, which is calculated from the average of control group.

inferior vena cava, avoiding hepatic portal system. After the intragastric administration stopped, serum E2 of intragastric group also subsided to a normal level. Altogether, uterine stent exhibited greater E2 delivery efficiency than oral adminiatration.

The trend of E2 alteration in left uterus (without stent implantation) was similar between intragastric group and stent group. E2 reached peak at 7 d post administration, and the concentration was 72.8 ± 14.583 pg/mL and 87.3 ± 9.604 pg/mL, respectively. After that, E2 concentration slowly descended; after 60 d, E2 declined to the control level (Fig. 5A, Supplementary Table S3). For the right side, however, stent implantation induced a boost in E2 concentration, reaching 2442.7 ± 2896.412 pg/mL in only 3 h post implantation. E2 concentration of the stent side kept an extremely high level during implantation. Nevertheless after removal of the stent, E2 quickly sloped to a physiological level (Fig. 5B, Supplementary Table S3). These results support the controllability of E2-releasing stent. The variance of two sides of uterine demonstrates the strong local E2 delivery efficacy of the stent.

3.3. Local safety of uterus after stent implantation

We compared the uterus specimen from the control group, and the 365 d sub-group from the stent group, to evaluate the long-term affects of stent implantation to uterine local safety. Gross examination revealed symmetrical uterine horns and smooth outer surface in the control group. Also, no evident wound or exterior abnormality was observed in the stent group, except for the right horn of one uterus, which was considered to be due to the surgical processes (Fig. 6 A). A higher average wet weight of right uterus horn was measured in the stent group. However, there was no significant difference (Fig. 6 B).

To find out whether E2-releasing stent caused intolerable morphological and/or functional changes in uterine, we harvested uterus histological sections from the control group and 365 d subgroup of the stent group. According to the H&E staining results, the stent-implanted side and the other side of the stent group uterus showed no apparent morphological difference with the control group. The endometrium was in a contact arrangement, with scattered glands and vessels. No obvious squamous metaplasia or endometrial adhesion occurred in any section (Fig. 6 C). We also used Masson staining to evaluate the extent of fibrosis in endometrium, which is a feature of endometrial adhesion or scar tissue. The sections did not present a sign of collagen deposition (Fig. 6D).

3.4. Stent did not form a high E2 level environment in remote organs in long term

To assess the safety of stent estradiol releasing for rodents, we examined the estradiol level in major organs, including hearts, livers, spleens, lungs and kidneys. We applied ELISA assay to measure the E2 concentration in the supernatant of organ specimen. For all the five organs, stent implantation or intragastric E2 administration did not induce remarkable or persistent rise in E2 concentration (Fig. 7, Supplementary Table S4). There were only minor fluctuations around the baseline, which was estimated according to the control group. In specific, there was no statistically significant elevation in heart E2 concentration after any form of E2 treatment. Besides, in liver, spleen, lung and kidney, E2 stent or intragastric administration generated slight alterations in E2 concentration, but the diverse was insignificant after 42 d. In addition, after exogenous E2 withdraw, the E2 concentrations in major organs maintained the same level with the control group. These findings indicate that E2-realeasing stent causes no E2 accumulation in major organs.

4. Discussion

To efficiently prevent occurrence of IUA after intrauterine operations, we combined the schemes of physical barrier and estrogen therapy, and designed a silicone rubber uterine stent system carrying E2. For the assessment of E2 releasing capacity and *in vivo* safety of this system, we implanted stents in rodent uterines, and measured E2 concentrations in blood, uterine and other organs at different time points. Our data demonstrate that the stent system continuously released E2 throughout the 60-day experimental period, and dramatically elevated serum and in situ E2 concentration, much more efficiently than intragastricly administration. After stent removal, serum and uterine E2 rapidly returned to normal level. Placement of stent did not obviously damage endometrium morphology. Also, there was no E2 accumulation in remote organs during stent implantation.

Estrogen is vital for endometrium regeneration. It binds to estrogen receptors, and then is delivered to the nucleus to stimulate a cascade of biochemical reactions. Estrogen receptors are widely expressed in endometrial epithelial and stromal cells [21]. E2 stimulates proliferation of endometrial epithelium cells by promoting pentose phosphate pathway metabolism, which provides materials for nucleotide synthesis [22]. Endometrial glandular growth requires

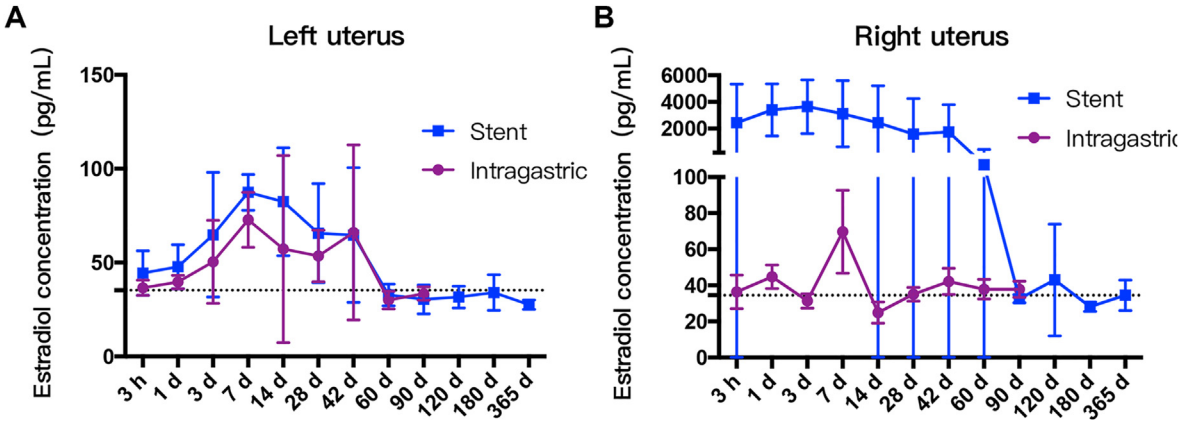

Fig. 5. E2 concentrations in uterine tissue. A. The curve illustrates cumulative release of E2 verses time for left uterus. The dotted line shows the E2 baseline of left uterine, which is calculated from the average of control group. B. The curve illustrates cumulative release of E2 verses time for right uterus. The dotted line shows the E2 baseline of right uterine, which is calculated from the average of control group.

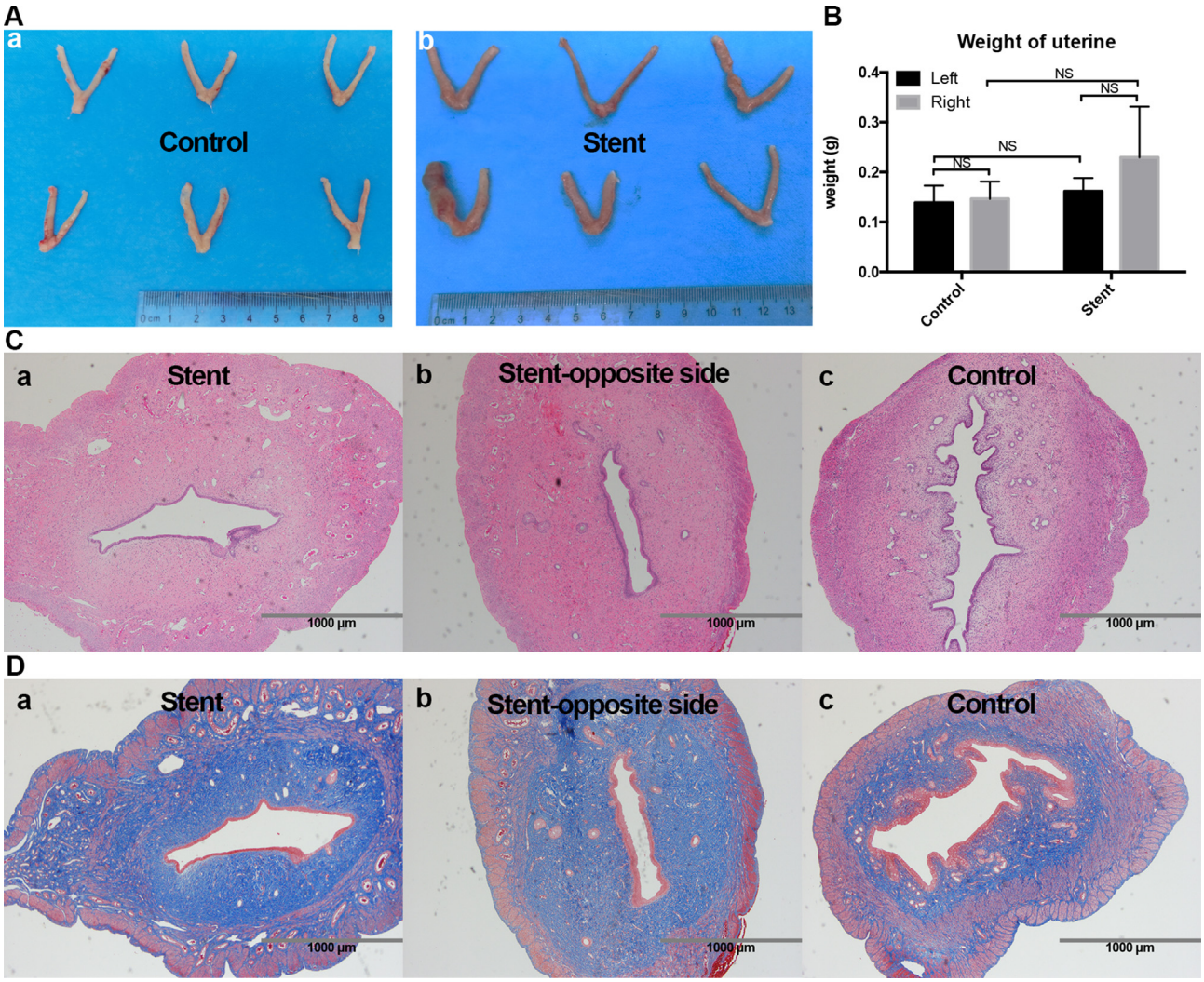

Fig. 6. Morphology of endometrium after stent implantation. A. Uterine specimen from the control group (a) and 365 d sub-group from the stent group (b) were similar in size and appearance in gross examination, except for one specimen in stent group, which was injured by surgical procedure. B. Weight of each side of the uterine from control group and 365 d sub-group of the stent group. n = 6; ns, n ≥ 0.05. C. H & E staining was applied in cross sections of uterines. The (a) right side of uterine in 365 d sub-group of stent group, which was implanted with stents, was compared with the (b) corresponding unimplanted side and the control group. D. Masson staining was applied in cross sections of uterines. The (a) right side of uterine in 365 d sub-group of stent group, which was implanted with stents, was compared with the (b) corresponding unimplanted side and the control group.

estrogen [23]. On the other hand, estrogens regulate endometrial angiogenesis by a paracrine route. E2 acts on the ERα+/ERβ+ endometrial stromal cells to stimulate cystathionine-β synthase expression, thus inducing H_2S production, which interacts with endometrial microvascular endothelial cells to enhance angiogenesis [24]. For the specific pathological change of IUA, several studies have shown that E2 acts as a fibrosis inhibitor in many diseases. Chronic use of E2 attenuates cardiac fibrosis by inhibiting Rho/ROCK/cofillin pathway [25]. Moreover, E2 counteracts TGF-β, thereby reducing collagen synthesis to decrease dermal fibrosis [26]. The mechanism of E2 inhibiting TGF-β signaling is most likely through promoting Smad 2/3 degradation [27]. E2 protection against ischemia-reperfusion injury-induced renal fibrosis is also exerted by inhibition of TGF-β type I receptor-SMAD pathway [28].

E2 reveals therapeutic values for IUA in clinical researches. Administration of estradiol sustains adequate endometrium by inducing endometrial proliferation [29,30]. Estrogen therapy is beneficial in patients with IUA regardless of stage of adhesions. Therefore, estrogen would be an efficacious ancillary treatment of adhesiolysis, and a helpful prevention scheme after intrauterine operations. It is reported that a 9 mg/d oral estradiol valerate before transcervical resection of adhesions was superior than a 3 mg/d dose in restoring menopause and recovering uterus shape [31]. This result indicates that the sufficient concentration of E2 ensures the efficiency of treatment.

However, orally taken estrogens are confronted with hepatic first-pass metabolism, thus oral or systemic administration could not induce satisfactory E2 concentration in situ at the injured uterine, which may reduce therapeutic effect [32]. This is in accordance with our findings: although the content of daily E2 by intragastric administration (approximately 80 μg for a rat) was larger than the loss of E2 for one day in a piece of stent (an average of 21 μg), E2 concentration in blood and the stent-implanted uterine of the stent group was much higher than the intragastric group. Furthermore, a high systemic E2 level might increase the risk of thrombosis and malignancy [33]. These drawbacks limit the clinical application of E2 in IUA prevention. Therefore, exogenous vaginal or intrauterine E2 supplementation is more effective than oral steroid therapy, circumventing the liver first-pass effect and promoting in situ E2 concentration [34].

To improve the therapeutic efficiency, and reduce the side effects of systemic E2 administration, researchers are exploring new

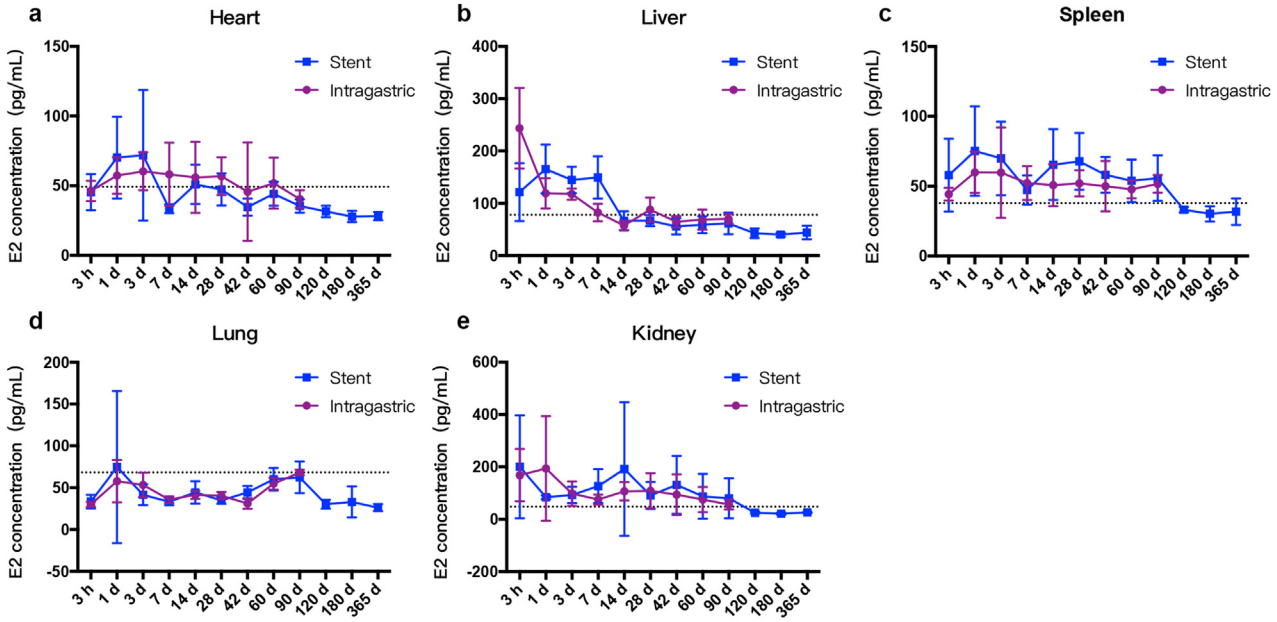

Fig. 7. E2 concentrations in organs. Plots of cumulative concentration of E2 verses time for the stent and intragastric administration in (a) heart, (b) liver, (c) spleen, (d) lung and (e) kidney are shown as mean ± SD. The dotted line shows the E2 baseline, which is calculated from the average of control group.

formulations of localized intrauterine delivery for IUA. Zhang et al. constructed a hydrogel-based sustained releasing system. E2 was encapsulated into the micelles of heparin-poloxamer to form a thermosensitive hydrogel (E2-HP hydrogel). The hydrogel could be injected into uterine lumens at a low temperature in a fluid state, and form three-dimensional network structure at body temperature *in vivo*. E2-HP-hydrogel effectively facilitated endometrium regeneration in IUA rats. Nevertheless, the hydrogel only retained a short time in uterine, and disappeared within a few days [35]. To solve this problem, the research team ameliorated the scaffold to a decellularized uterus derived nanoparticle-composed aloe/poloxamer hydrogel. The novel hydrogel prolonged E2 release to more than a week, and significantly increased uterine morphological recovery while decreasing fibrosis rate [36]. Another approach loaded E2 in poly lactic-coglycolic acid (PLGA) microspheres (E2-MS), then dispersed the microspheres in human amniotic extracellular matrix (HAECM) stents. E2-MS-HAECM scaffolds showed a sustained release of E2 for 21 days *in vitro* [37].

Nevertheless, these gelatinous scaffolds might easily be expulsed from human uterine. Considering this, we conceived of a solid phase E2-releasing stent with rigidity, so that the stent would take advantage of two anti-IUA schemes at the same time. Our stent is composed of silicone rubber, which is widely used in medical implants for its fine biocompatibility and high chemical stability [38,39]. The intrinsic property of our silicone rubber stent is semiflexible with stiff architectures, so that the stent can be shaped into the structure of IUDs. In this way, the stent could help preventing adhesion by separating the anterior and posterior uterine walls. Since the stent system achieved a high E2 concentration in blood and uterine, we can advance the system by reducing the E2 dosage in stent.

IUDs have been utilized in post-operative IUA prevention, and could reduce over 30% recurrence rate after hysteroscopic adhesiolysis [40]. Despite the success IUDs have achieved in clinical applications, they are not risk free. Complications including expulsion, malpositioning and uterine perforation raise concerns [41]. Furthermore, copper IUD insertions exhibit poor biocompatibility. As a consequence, they could induce infection and inflammation [42]. Throughout the study, no perforation on uterine or stent-uterine wall adhesion was observed when we removed the stents by surgery. According to the morphology experiments, we did not observed inflammation infiltration or fibrosis scars in the endometrium of stent group (Fig. 6). These results indicate the safety of silicone rubber stents, which can be further improved to accommodate the shape of human uterine cavity.

In this work, we constructed a controlled-release system with silicone rubber stent incorporated with E2. The stent system notably raised local E2 concentration in uterine, without injury to endometrium or E2 residual after treatment. Based on its fine efficiency and safety, this stent system could have a potential clinical application in endometrial regeneration.

5. Conclusions

The silicone rubber E2-releasing uterine stent provides both physical barrier and high concentrations of E2 to local endometrium in long term, therefore it can be a good treatment option for IUA.

Declaration of competing interest

The authors declare that they have no known competing interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.reth.2022.10.001.

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