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Reversible Mechanical Contraception and Endometriosis Treatment Using Stimuli-Responsive Hydrogels

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Female sterilization via fallopian tube ligation is a common procedure; However, after the operation, over 10% of women seek re-fertilization, which is frequently unsuccessful. In addition, there is evidence that fallopian tubes contribute to the spread of endometriotic tissue as they serve as channels for proinflammatory media entering the abdominal cavity via retrograde menstruation. Here, stimuli-degradable hydrogel implants are presented for the functional, biocompatible, and reversible occlusion of fallopian tubes. The hydrogel implants, designed with customized swelling properties, mechanically occlude fallopian tubes in a high-performance manner with burst pressures reaching 255–558 mmHg, exceeding normal abdominal pressures (95 mmHg). Their damage-free removal can be achieved within 30 min using near-visible UV light or a glutathione solution, employing a method akin to standard fallopian tube perfusion diagnostics. Ultrasound-guided implant placement is demonstrated using a clinical hysteroscope in a human-scale uterus model and biocompatibility in a porcine in vivo model. Importantly, the prevention of live sperm as well as endometrial cell passage through blocked fallopian tubes is demonstrated. Overall, a multifunctional system is presented that constitutes a possible means of on-demand, reversible contraception along with the first-ever mechanical approach to abdominal endometriosis prevention and treatment.

1. Introduction

Fallopian tubes are essential to a woman's reproductive health. They are essential for the movement of oocytes and sperm between the uterus and the ovaries, as well as facilitate fluid exchange between the uterus and the peritoneal cavity. Due to their central role in the fertilization process, female sterilization, achieved by the ligation, occlusion, or the removal of fallopian tubes, is commonly performed, with procedures such as these ranking as the second most used contraceptive method in the United States.[1] These procedures prevent the passage of oocytes down the fallopian tubes and block the passage of sperm cells, averting fertilization.[2] Non-hormonal contraceptive methods have swiftly gained traction due to their minimal side effects. Despite the frequent use and efficacy of tubal sterilization, more than 10% of American women that undergo this treatment regret

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their decision to be sterilized. The most commonly reported reasons for sterilization regret are subsequent gynecological or menstrual problems and the desire to have children[3]; However, surgical reconnection of the ligated tubes is a complicated and expensive procedure with no guarantee of success.[4] Despite their caveats, permanent contraceptive methods involving the disruption of fallopian tubes retain popularity due to their effectiveness in preventing unintended pregnancies.[5,6] Alternatively, hysteroscopic tubal sterilization techniques have presented women with less invasive approaches for permanent contraception. One of those devices was the Essure device, a hysteroscopic micro-insert, which was placed at the proximal part of both fallopian tubes inducing fibrosis and consequently leading to tubal occlusion. The transcervical application of such devices enabled women to undergo sterilization in an easy, safe, and cost-effective procedure, performed within 15 min in an outpatient procedure at a gynecologist's practice and without the use of general anesthesia[7,8]; However, Essure was removed from the market in 2018, as more than 32% of patients developed gynecological complications and ≈15% of these requested surgical removal.[9,10] Nevertheless, minimally invasive, hysteroscopic occlusion of the fallopian tubes presents an appealing alternative to conventional laparoscopic sterilization, as demonstrated by the initial success of Essure. In recent years, novel materials for the occlusion of fallopian tubes have been proposed. For example, Femasys, Inc. is developing a degradable biopolymer liquid (FemBloc), which can be injected into the fallopian tubes through a catheter-based delivery system causing scarring and tubal occlusion within three months after injection[6]; However, these strategies have led to gynecological complications in some patients and do not address the need for a safe and effective method for re-fertilization. Moving toward effective but reversible sterilization materials, Wang et al. proposed a long-lasting, male contraceptive method deploying ultrasound-induced degradable hydrogels.[11] In a different approach, Subramanian et al.[12] developed a non-hormonal tubal contraceptive implant using a hydrogel formulation of styrene-cis-butylene-based styrene maleic anhydride in dimethyl sulfoxide. This hydrogel lowers the pH around sperm, disrupting the acrosome and reducing sperm viability, thus preventing fertilization. Abdala et al.[13] utilized ethylene–vinyl alcohol copolymers in a rabbit model to create a biocompatible, spongy plug in the fallopian tubes, effectively preventing fertilization. This copolymer solidifies upon contact with aqueous media, sealing the lumen. Although successful in blocking fertilized egg injection sites in rabbits, there were variations in occlusion, fibrosis, and inflammation. Shveiky et al.[14] tested PF-88, a reverse thermo-responsive polymer, and found it effective in reversibly blocking rabbit fallopian tubes for up to twenty eight weeks. In general, polymer-based materials with on-demand degradation could present a valid option for the functional occlusion of the fallopian tubes and introduce a novel method of long-acting, reversible, and non-hormonal contraception for women; However, none of the existing approaches has leveraged the swelling of hydrogels to achieve gentle and reversible but highly effective tubal occlusion.

Interestingly, the occlusion of the fallopian tubes has the potential to introduce considerable additional benefits, including the first ever mechanical treatment for preventing endometrial lesions in the fallopian tubes and the abdominal cavity. Endometriosis is a chronic, gynecological disease defined by the presence of endometrial-like tissue outside of the uterus, predominantly found in the peritoneal cavity.[15,16] ≈10% of all women of reproductive age are affected by this devastating condition.[17] Major symptoms include chronic pelvic pain, dysmenorrhea, dyspareunia, and infertility.[18] Despite its substantial impact on the physical, sexual, and mental well-being of the affected women, endometriosis still lacks awareness in the general population and symptoms are often downplayed.[18–20] Due to a symptom "threshold" needing to be reached before evaluation by explorative laparoscopy, average delays of 8–11 years between symptom onset and diagnosis have been reported.[21,22] Existing therapies, such as surgical removal of endometrial lesions, anti-inflammatory medication, and/or hormonal treatment, have variable efficacy and are often accompanied by additional negative side effects.[18] Notably, the surgical removal of endometrial lesions on vital abdominal organs is delicate and associated with serious complications, including intestinal injury and leaking of microbially active gastrointestinal fluid into the abdomen, putting the patients at risk for sepsis. Aggravating the issue, ≈50% of women suffer from recurrent symptoms within several years, independent of treatment approach.[23–25] While various hypotheses on the origin of endometrial lesions have been postulated, Sampson's theory of retrograde menstruation is the oldest and remains the most widely accepted.[26] It proposes that menstrual dissemination and reflux of endometrial cells through the fallopian tubes, or so-called retrograde menstruation, is the major cause of endometriosis.[26,27] This theory is supported by studies showing increased risk of endometriosis in women with short menstrual cycles or obstructed menstrual flow; However, the exact pathogenesis of endometriosis remains unclear.[17,18] Gaining a more profound understanding of its etiology is considered crucial for the development of novel therapies as there are currently no curative treatments available.[18] Interestingly, while the pathogenesis is not fully understood, therapies that can prevent retrograde menstruation, such as fallopian tube occlusion, have the potential to not only improve our understanding of the condition, but also drastically improve the livelihood of women suffering from endometriosis.

Here, we present two on-demand, stimuli-removable hydrogel systems for the reversible occlusion of fallopian tubes toward reversible sterilization and potential treatment of abdominal endometriosis. The proposed hydrogel systems are composed of two different acrylamide-based polymers crosslinked with either the photolabile molecule poly(ethylene glycol) di-photodegradable acrylate (PEGdiPDA)[28] or the disulfide crosslinker N, N'-bis(acryloyl)cystamine (BAC), creating hydrogels that are degradable on a clinically relevant time scale by near-visible UV light or any disulfide reducing agents, such as the biocompatible glutathione, respectively.[29] The simplicity and ease of use of the hydrogel systems is demonstrated in a clinical setting using a custom-made, human-scale uterus model along with ultrasound-guided hydrogel placement using a hysteroscope. The ability of the swollen hydrogels to effectively block fallopian tubes in a reversible manner, yet biocompatible way, is demonstrated based on burst pressure experiments[30] the imaging of fluid passage using an echogenic contrast agent and the proof of concept implantation of a hydrogel system in the fallopian tube of a piglet for three weeks in vivo are showcased. Most

importantly, in a proof-of-concept experiment, endometrial cell passage is prevented through hydrogel-obstructed fallopian tubes shown in a custom-designed endometrial flow system with lesion forming endometrial cells.[31] The novel material systems presented here have the potential to improve the options for women seeking non-hormonal, reversible birth control without permanent tube occlusion. Additionally, they introduce a new purely mechanical treatment and prevention option for abdominal endometriosis.

2. Results and Discussion

2.1. Design of Stimuli-Removable Hydrogel Systems

The functional occlusion of the fallopian tube using stimuli-degradable hydrogels not only blocks the passage of sperm and oocytes, but also prevents the passage of endometrial cells. This inhibits fertilization and the formation of endometrial plugs in the peritoneal cavity, respectively (**Figure 1a**). For a suitable fallopian implant design, two distinct but chemically related compositions were investigated. The chemical composition of the stimuli-degradable hydrogels is illustrated in Figure 1b. Both systems consisted mainly of the superabsorbent (poly(2-acrylamido-2-methyl-1-propanesulfonic acid) sodium salt (PAMPS) and poly(N-(2-hydroxyethyl) acrylamide) (PNHEA).[32] PAMPS provides access to relatively high swelling ratios,[33] which are required for successful tissue compatible tubal blockage and subsequent efficient, stimuli-induced degradation. NHEA monomers were added to prevent excessive swelling and instability of fully hydrated gels while providing additional biocompatibility.[34] FT-IR spectroscopy measurements of as-prepared PL and TD show characteristic PAMPS peaks at 1039 cm^{-1} and 1183 cm^{-1} (dotted lines) for both gel types (Figure S1, Supporting Information).[35] Two types of hydrogels with distinct but "doctors' office" compatible degradation mechanisms, light versus reduction, were designed by utilizing different crosslinkers to form the hydrogels. The two different degradation mechanisms were explored to ensure degradation, and thus removal of the hydrogels could occur on clinically relevant time scales and explore how the use of different crosslinkers altered the swelling and mechanical properties of the resulting hydrogels.

First, the degradation of the different hydrogels was assessed to ensure degradation occurred on a clinically relevant time scale. Photolabile hydrogels (PL-Gel) were formed with poly(ethylene glycol)-based molecule PEGdiPDA[28] as a crosslinker in a 40 wt% mix of PAMPS and PNHEA. Degradation of the fully swollen PL-Gel was achieved by light irradiation (λ = 365 nm) with a medical optical fiber (I_0 = 40 mW cm^{-2}) within < 30 min. Likewise, thiol degradable hydrogels (TD-Gels) were formed using a disulfide crosslinker, BAC, in a 25 wt% PAMPS and NHEA mix. Exposure to disulfide reducing agents, including biocompatible glutathione (GSH)[29] was achieved using a common fallopian tube perfusion system and resulted in degradation of a TD-Gel within 30 minutes (c_{GSH} = 200 mM). In both cases, complete hydrogel degradation was achieved either using a clinically relevant light dosage (i.e., 10 – 130 J cm^{-2} for UVA1 light applied through skin[36]) for photolabile hydrogels or within a clinically relevant time scale (i.e., 20–30 min characteristic duration of a typical overall fallopian tube sperm perfusion procedure[37]).

After confirming the degradation of the two different formulations on a clinically relevant time scale, hydrogel placement was assessed using an application technique similar to the surgical placement of the Essure device. Since the stimuli-responsive hydrogels were designed to be soft ($E \approx$0.1 to 2 kPa) in their fully swollen state and block the fallopian tubes by functional occlusion rather than by induction of fibrosis, a slightly modified application method was utilized (Figure 1c). Tube-shaped hydrogels were first formed and vacuum dried. These as-prepared hydrogel articles were loaded into a catheter and inserted into the fallopian tube (ex vivo) through the working channel of a hysteroscope. The hydrogel articles were pushed through the catheter and placed at the proximal part of both fallopian tubes (hysteroscopic insertion). Upon tissue contact, the hydrogel article immediately started to swell, increasing in size to fill the fallopian tube. Within a few hours, the hydrogel reached its swelling plateau (Figure S2, Supporting Information), effectively blocking the fallopian tube and preventing sperm and endometrial cells from passing (tubal occlusion). At this point, if reversal of tubal blockage was desired, the hydrogel could be degraded using light (PL-Gel) or thiol-containing fluids (TD-Gel). As described above, both removal methods can be applied easily in a minimally invasive procedure through the working channel of a hysteroscope resulting in refertilization.

2.2. Characterization of Stimuli-Removable Hydrogel Systems

In situ swelling capacity and swelling kinetics for PL- and TD-Gels were estimated by full immersion of hydrogels in simulated oviduct fluid (SOF) ex situ (**Figure 2a**). Hydrogel weight was measured at different time points and swelling ratios were calculated. The swelling plateau of stimuli-removable hydrogels was reached within 4–6 h, resulting in a final swelling ratio of 12 (PL-Gel) and 16 (TD-Gel). The higher swelling equilibria of TD-Gels is attributed to its lower polymer weight fraction (25 wt% vs 40 wt%) and lower crosslinker concentration (1.31 mM vs 8.36 mM) compared to PL-Gels; However, since measurements were taken at full immersion in fluid (50 µL gel in 5 mL SOF) and without any compressive forces acting on the gel, a slower in situ swelling rate is expected as human tubal fluid production is limited and strongly dependent on the day of the menstrual cycle. Typical daily fluid production ranges from 0.3 mL to 9.6 mL with its peak 1–2 days before ovulation.[38] Therefore, implantation a few days prior to ovulation is recommended to ensure high swelling rates and prevent early dislocation of hydrogels. As-applied hydrogels were swollen in human peritoneal fluid for 24 h to assess the stability of PL and TD-Gels in relevant human biological fluid. Stability of fully hydrated hydrogels was quantitatively assessed by visual analysis and observed to be stable over the complete observation period (21 days). Long-term exposure to simulated oviduct fluid showed that the implants remained mechanically intact and stable for more than six months, indicating potential for long-term implantation. To assess as-applied hydrogel implant characteristics, scanning electron micrographs of a dry TD-Gel cross-section were recorded (Figure 2b). A porous network was observed without apparent differences between different regions of the implant (center or periphery), which is ideal for ensuring uniform hydrogel swelling.

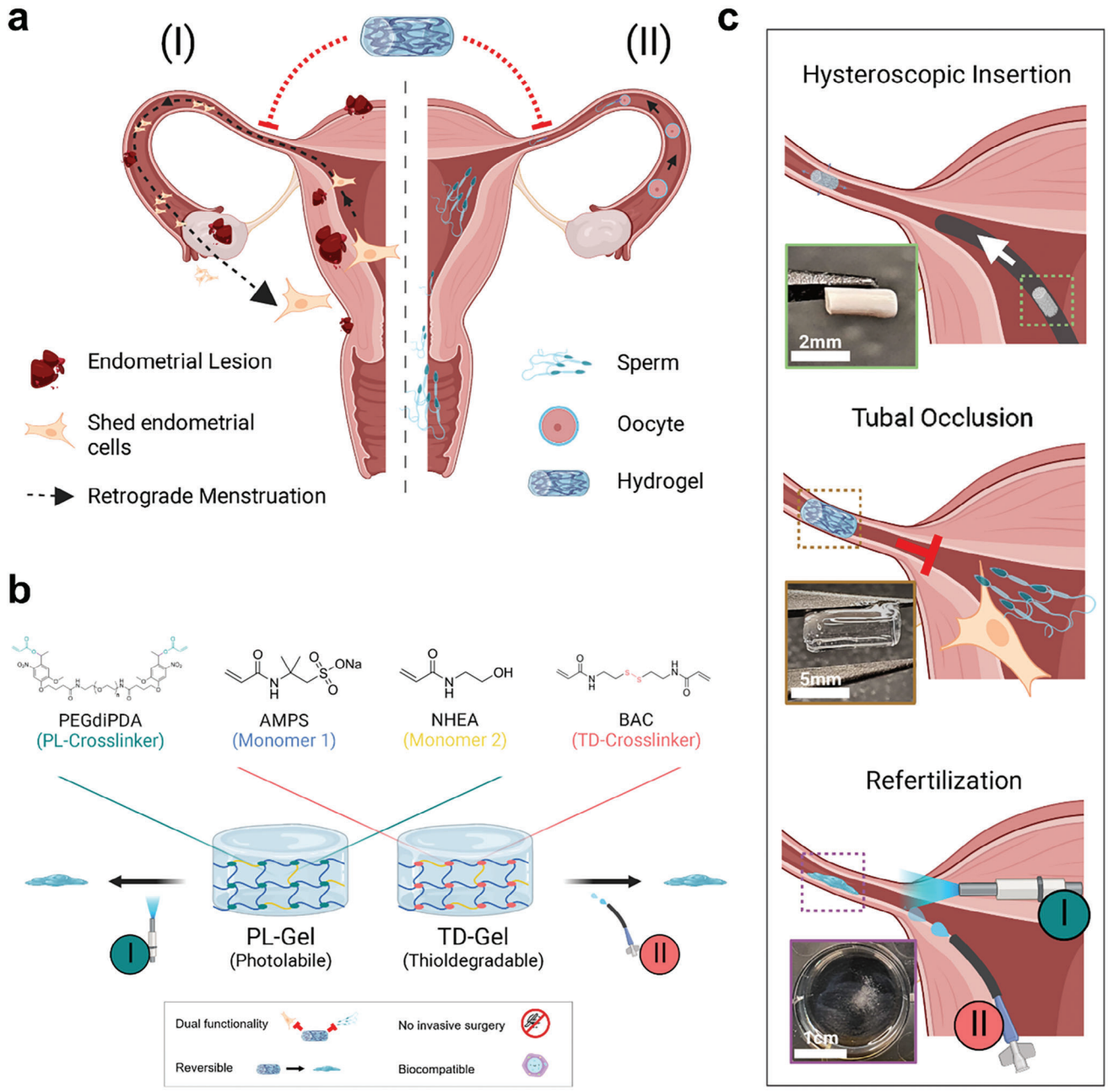

Figure 1. a) Illustration of functional fallopian tube occlusion serving two main purposes. (I) Endometriosis protection by preventing retrograde menstruation. (II) Contraception by functional occlusion through the hydrogel system averts passage of sperm and oocytes through the fallopian tubes and prevents fertilization. b) Formulation of two degradable hydrogel systems based on AMPS (2-acrylamido-2-methyl-1-propanesulfonic acid) and NHEA (N-(2-hydroxyethyl) acrylamide) along with main advantages of the gels. Left: Photolabile (PL) hydrogel crosslinked with PEGdiPDA. Right: thiol-degradable (TD) hydrogel crosslinked with BAC. c) Schematic representation of stimuli removable hydrogel platform at different stages of use (placement using a hysteroscope (top), tubal occlusion (center) and removal (bottom)). Figure created using Biorender.

Next, the viscoelastic properties of stimuli-degradable hydrogels and porcine oviduct sections were assessed using rheological analysis (Figure S3, Supporting Information). Average measured values are set into perspective by comparing them to literature data (Figure S4, Supporting Information).[39] Storage moduli of porcine fallopian tubes were approximately two times lower as fully swollen PL-Gels, however, more than ten times higher than TD-Gels (Figure 2c,d). As expected, based on polymer concentration and hydrogel swelling, fully hydrated TD-Gels were softer than PL-Gels. As both hydrogels were of a similar modulus compared to fallopian tube tissue, significant distortion of the fallopian tube due to excessive hydrogel swelling is unlikely. Further rheological measurements were performed to quantify degradation kinetics of the swollen PL-Gel upon light exposure

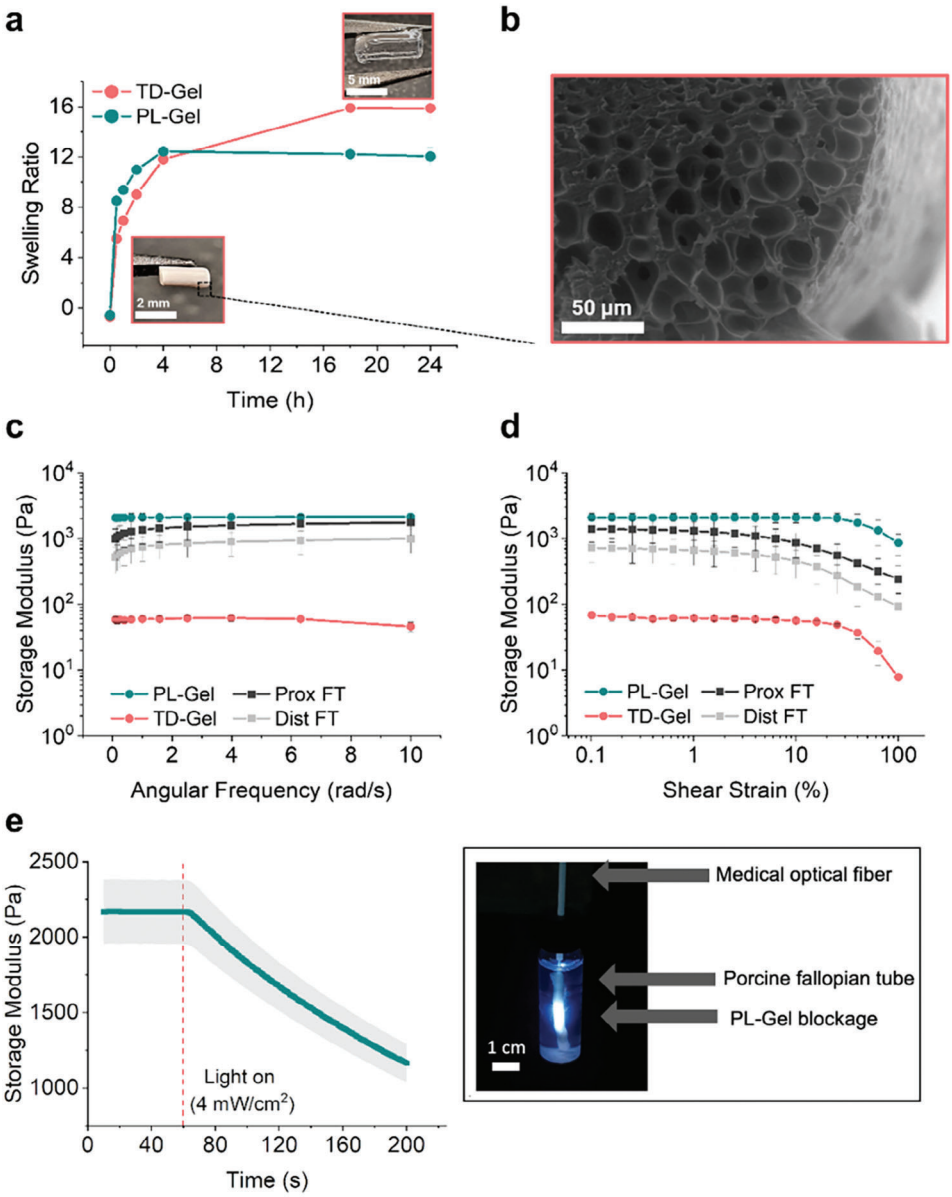

Figure 2. Material properties characterization. a) Swelling behavior of photolabile (PL) and thiol-degradable (TD) gels in simulated oviduct fluid (SOF). b) Scanning electron micrograph of an as-applied TD-Gel. c) Storage moduli of PL and TD gels as a function of frequency and d) shear strain. e) Change in storage modulus over time upon light exposure indicating gel degradation. Photograph of medical optical fiber degrading hydrogel-blocked, porcine fallopian tube in a background of simulated fallopian tube fluid. Data displayed as mean ± standard deviation. Measurements were performed in triplicates (N = 3).

(Figure S4, Supporting Information). The first-order effective degradation rate constant was calculated (k_{eff}/I_0 = 24 cm^2 mW^{-1} s^{-1}) and indicates a higher rate of degradation for swollen PEGdiPDA crosslinked hydrogels compared to degradation rates reported for non-swollen PEG-based photolabile hydrogels (k_{eff}/I_0 = 5–10 cm^2 mW^{-1} s^{-1}).[40,41] This is expected, since after swelling of the PL-Gels the photoactive crosslinker is diluted compared to its non-swollen state leading to less light attenuation and fewer crosslinks per unit volume, suggesting a relationship between swelling behavior and degradation kinetics. These faster degradation kinetics are advantageous for the desired application and can afford full hydrogel degradation using a medical optical fiber setup (Figure 2e). Using the optical fiber setup and UV exposure of 30 min for full decrosslinking, we did not observe any damage to host tissue (Figure S4, Supporting Information). While the light irradiation conditions utilized in this work are considered safe,[42] the fallopian tube tissue integrity and fertility are to be further monitored carefully prior to clinical use. Degradation by irradiation with blue light (λ = 405 nm) may be explored as an alternative as the nitrobenzyl groups of the PEGdiPDA are known to degrade at this wavelength.[40] Overall, the conditions for degradation utilized here were selected to

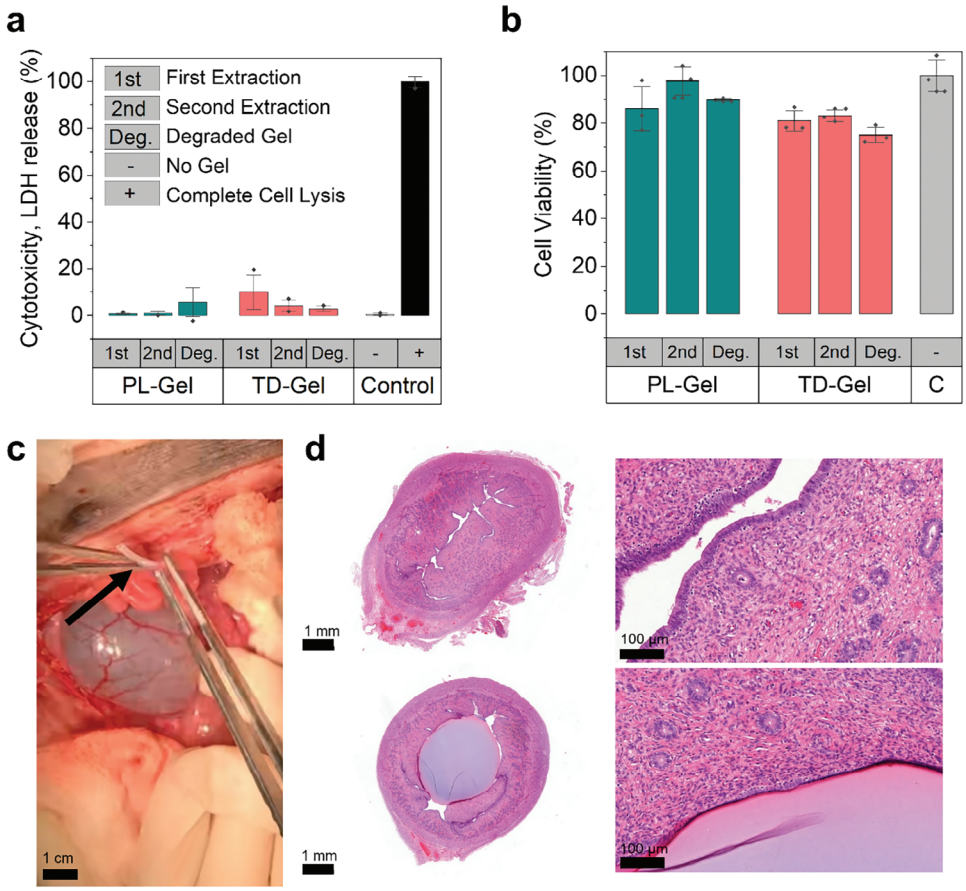

Figure 3. Hydrogel cytocompatibility and tissue compatibility assessment. a) Lactate dehydrogenase (LDH) release cytotoxicity assay of human fibroblasts cells cultured in cell medium subjected to different states of PL and TD hydrogel particles. Cell culture medium and medium containing 0.1% TritonX were used as negative (grey) and positive (black) controls, respectively. b) Viability of human fibroblasts after 24 h of incubation with hydrogel conditioned media. Cell culture experiments were carried out in triplicate (N = 3). c) Surgical placement of hydrogel into pig fallopian tubes in vivo for long term biocompatibility assessment. d) H&E staining of porcine oviduct sections (isthmic region). (top) patent (non-blocked) oviduct. (bottom) oviduct blocked with TD-Gel for three weeks in vivo.

minimize the chance of damage to the host. Note also that the glutathione degradable option presented here may be used either alone or in combination with light, thus further reducing the irradiation dose. Compared to the permanent fallopian tube scarring introduced by alternative sterilization methods, the above conditions likely offer relevant benefits with regard to refertilization potential. Overall, both the PL- and TD-Gels have high swelling ratios that can lead to fallopian tube occlusion with mechanical properties that are not anticipated to lead to significant distortion of the fallopian tube and can readily be removed using a tissue-compatible trigger.

2.3. Cytocompatibility with Human Cells and Biocompatibility In Vivo

Biocompatibility of the stimuli-responsive hydrogels throughout the entire procedure associated with their placement, was assessed using a lactate dehydrogenase (LDH) release assay of fibroblast cells incubated with hydrogel conditioned media and histology of fresh porcine fallopian tubes (**Figure 3**). Cytotoxicity of the as-applied (1st Extraction), swollen (2nd Extraction), and degraded (Deg.) hydrogels was investigated by immersion of hydrogels in (2 mL) cell culture media and subsequent cell culture of fibroblasts with the conditioned media (Figure 3a). The first extraction represents toxicity of hydrogels immediately after placement in the fallopian tubes and the second extraction characterizes the cytotoxic effect of hydrogels after full hydration. Furthermore, to investigate possible cytotoxic effect of degradation products, hydrogels were degraded in media after reaching equilibrium swelling. Lactate dehydrogenase (LDH) release from fibroblasts was measured at different culture durations after incubation with conditioned media (t = 4 h, 24 h, 48 h). The data shows negligible cytotoxicity of PL-, TD-Gels and their degradation products and an LDH release comparable to that of fibroblasts cultured in fresh, unconditioned cell medium. Additionally, cell viability using a metabolic activity assay (CellTiterGlo) was assessed after 24 h of incubation with conditioned media and observed to be similar to control cells (Figure 3b).

To assess tissue compatibility of the implantation and expansion of PL- and TD-Gels, histological analysis of non-blocked and blocked porcine fallopian tubes was performed after in

vivo implantation for three weeks in a piglet model. Successful placement of the hydrogel was achieved using a small incision in the fallopian tube (Figure 3c). After three weeks in vivo the hydrogel was identified and histological analysis completed (Figure 3d).There, the main tissue layer of concern is the inner mucosa, which is composed of ciliated and non-ciliated secretory epithelial cells responsible for tubal fluid production and gamete transport through the fallopian tubes.[43,44] Functional impairment of the inner lining of the fallopian tubes may result in infertility and ectopic pregnancies.[45] The longitudinal folds of the mucosal tissue (plicae) are clearly visible in the histological H&E stained fallopian tube sections (Figure 3d; Figure S5, Supporting Information). Note that the various processing steps of the occluded fallopian tube sections have caused the hydrogel to shrink slightly; However, no apparent damage or deformation of the plicae is visible in the occluded tubes when compared to non-blocked (patent) tubes. In addition, no rips or significant distortions of the smooth muscle layer surrounding the mucosa were observed. The hydrogel showed good contact with fallopian tube and no observable signs of degradation. Additionally, according to the blinded analysis by a certified pathologist, there was no histological difference between the controls and the sample with the implant except some accumulation of fluid and macrophages in the lumen. Importantly, no histological findings indicative of a foreign body reaction could be observed. Together, these results demonstrate that the hydrogels can be placed within the fallopian tube and maintained at the site of interest over several weeks indicating their potential for tube occlusion.

2.4. Surgical Application and Ultrasound Guided Placement

To ensure hydrogels can be placed within the fallopian tube using common gynecological techniques, the surgical application of as-applied hydrogels was simulated in a human-scale acrylamide/gelatin uterus model (**Figure** 4a; Figure S6, Supporting Information). The model was designed to mimic surgical access of a hysteroscope through the vagina and cervix. The vagina was represented by the 10 cm long and ≈3 cm wide rod-shaped cavity and the cervical canal was mimicked by the narrow section between the anatomical uterine cavity and the vagina (Figure 4a). The functional occlusion of the fallopian tubes was simulated using surgical tools available in a standard gynecologist's operating room (Figure 4b). Images taken during the simulated insertion of the hydrogels into the fallopian tubes of the model are shown in (Figure 4c).

The orifice of the fallopian tubes could be successfully identified with the hysteroscope and was followed by insertion of the catheter into the fallopian tube. After catheter placement, a small Teflon tube (outer diameter, OD = 0.8 mm) was inserted into the catheter and a small version of the as-prepared hydrogel (D = 0.8 mm) was pushed out through the catheter and into the fallopian tube using a Teflon tube to push the gel through the catheter and into the lumen of the fallopian tube. Blockage of the tubes with a PL-Gel was monitored using ultrasound imaging (Figure 4d). The fallopian tubes and the uterine cavity are clearly visible and blocked and non-blocked tubes can be distinguished by the change in contrast observed within the tube. These model applications demonstrate the potential utility of the system presented here to be inserted successfully into the fallopian tubes using common gynecological equipment.

2.5. Fallopian Tube Occlusion and Re-Fertilization

Constant physiological fluid movement and physiological pressures within the fallopian tubes have the potential to impact the long-term stability of the hydrogel's placement. The effective blocking of the tube was therefore further confirmed by ultrasound imaging using an ultrasound contrast agent (ExEm foam). Effective blocking of the contrast agent by the implant was observed by ultrasound (**Figure** 5a). Functional hydrogel blockage and stimuli-responsive removal was further assessed using burst pressure measurements on blocked, unblocked, and control porcine fallopian tube sections (Figure 5b; Figures S7–S9, Supporting Information).[30,46] For the expulsion of fully swollen hydrogels placed within the fallopian tube, average pressures of 558 mmHg (PL-Gels) and 255 mmHg (TD-Gels) were necessary. This significantly exceeds the highest measured intra-abdominal pressure of 95 mmHg as well as normal physiological pressures of 50 mmHg observed in the fallopian tubes.[47,48] Burst pressure of in situ swollen PL-Gels decreased to 198 mmHg upon exposure to 30 min of light irradiation (λ = 365 nm, I_0 = 40 mW cm^{-2}) with an optical medical fiber. Burst pressure of TD-Gels decreased to 67 mmHg upon exposure to a 0.2 M glutathione solution (dissolved in PBS, pH adjusted to 7.2) and continuous flow for 30 min. In TD-unblocked samples, measured pressures were very similar to the non-blocked control suggesting full degradation of two of the TD-Gels. The burst pressure data indicates that PL and TD-Gels are both valid systems for the blockage of fallopian tubes as they exceed maximal intra-abdominal pressures measured; However, even though significant decreases in the burst pressure were observed upon hydrogel exposure to stimuli, complete degradation of the hydrogels was only achieved for the TD-Gels and in not for the PL-Gels. In fallopian tubes blocked with non-stimuli responsive hydrogels (PL-C and TD-C), no significant difference between blocked and unblocked tubes was seen after exposure to degradation stimulus (Figure 5b). These measurements confirm that the observed significant decreases in burst pressure after light or reducing agent exposure are indeed related to the breaking of crosslinks in the macromolecular network. Once these crosslinks are reduced to under 50%, the mechanical integrity of the gels is no longer given, and the degradation products are washed out from the fallopian tubes by physiological fluid circulating. Based on the cytocompatibility assessments performed, no adverse effects are to be expected and the degradation products are eventually excreted transvaginally.

2.6. Endometriosis Model

As a final proof-of-concept, an in vitro model was developed to assess if the hydrogel could prevent retrograde menstruation and live sperm passage. Endometrial cells were flown through either a non-blocked or PL-Gel blocked fallopian tube using a setup similar to that used for burst pressure measurements. The passage of endometrial cells through the obstructed fallopian tubes were assessed (Figure S10, Supporting Information). First, the exposure

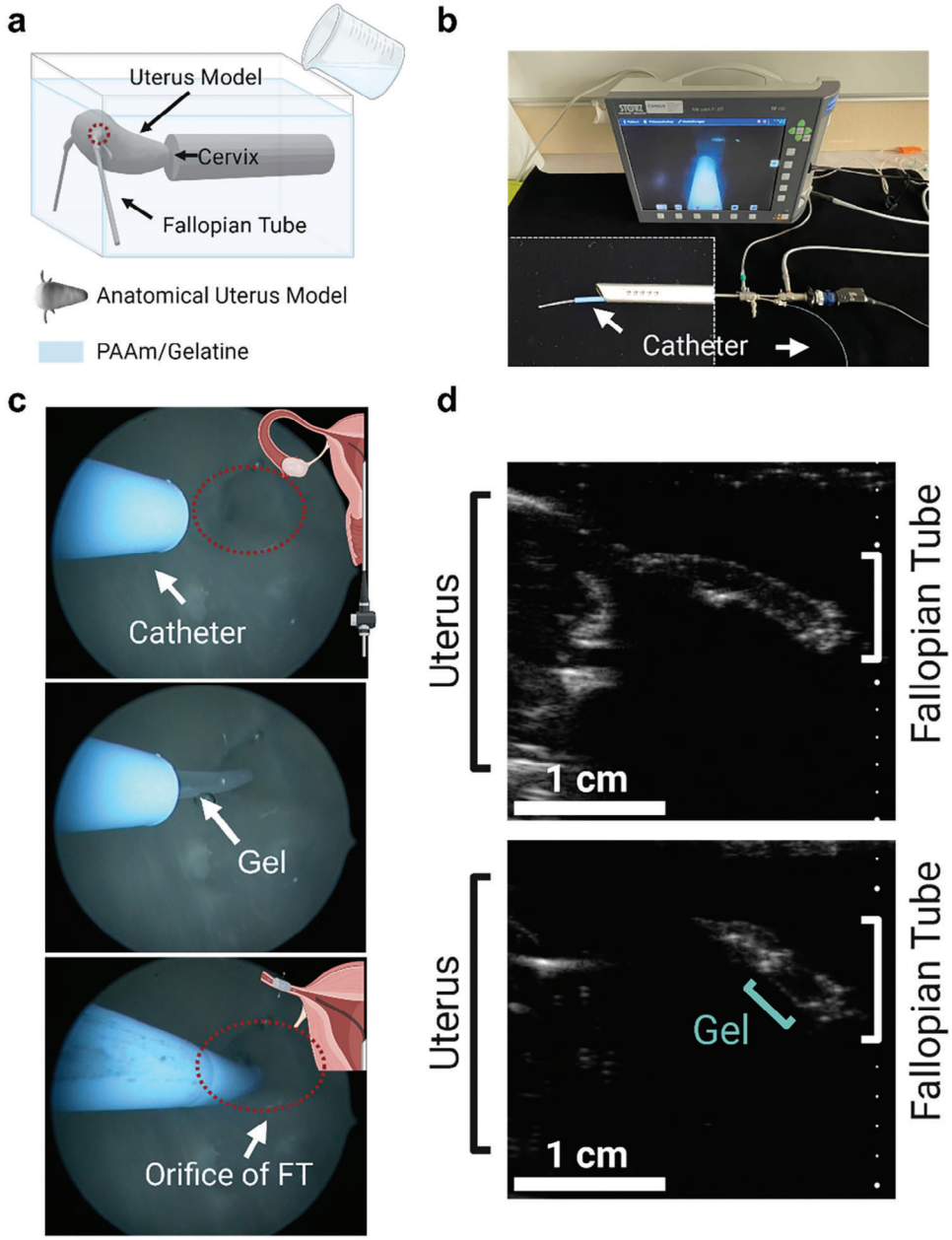

Figure 4. Surgical application of hydrogel system in a human-scale uterus model. a) Schematic of acrylamide/gelatin uterus model developed to mimic the surgical application of PL and TD hydrogels. b) Tools needed for hysteroscopic insertion of gels into the fallopian tubes (Hysteroscope (labelled in figure by white arrow), open-end ureteral catheter, LED Monitor). c) Hysteroscope images taken during simulated placement of hydrogels in the fallopian tubes of the acrylamide/gelatin uterus model. d) Ultrasound images of uterus model. Top: Patent fallopian tube. Bottom: Fallopian tube occluded with hydrogel (D = 4 mm).

of lesion-forming endometrial cells to the flow systems cyclical increases and decreases in pressure (Δp = 50–60 mmHg, freq. ≈1.6 cycles/min) was determined to not cause significant cell damage in the tested timeframe (30 min) compared to static control (Figure S10, Supporting Information). After ascertaining that the setup did not cause cell damage, cells were passed through non-blocked and blocked fallopian tubes. Excitingly, the placement of the PL-Gel within the fallopian tube led to no cells being able to migrate through the tube (n = 3), while ≈40 000 out of 1 million available cells were able to pass through the non-blocked fallopian tube (n = 3) over a period of 30 min (Figure 5C; Figure S11, Supporting Information). Microscopy images were utilized to qualitatively confirm the lesion forming nature of the endometrial cells. In addition, when the perfusion time was extended to 4 h (this time using fixed cells to avoid cell lysis), no endometrial cells were found to pass through the fallopian tube (Figure S11, Supporting Information). These analyses again confirmed that no cells passed through the occluded tube and no

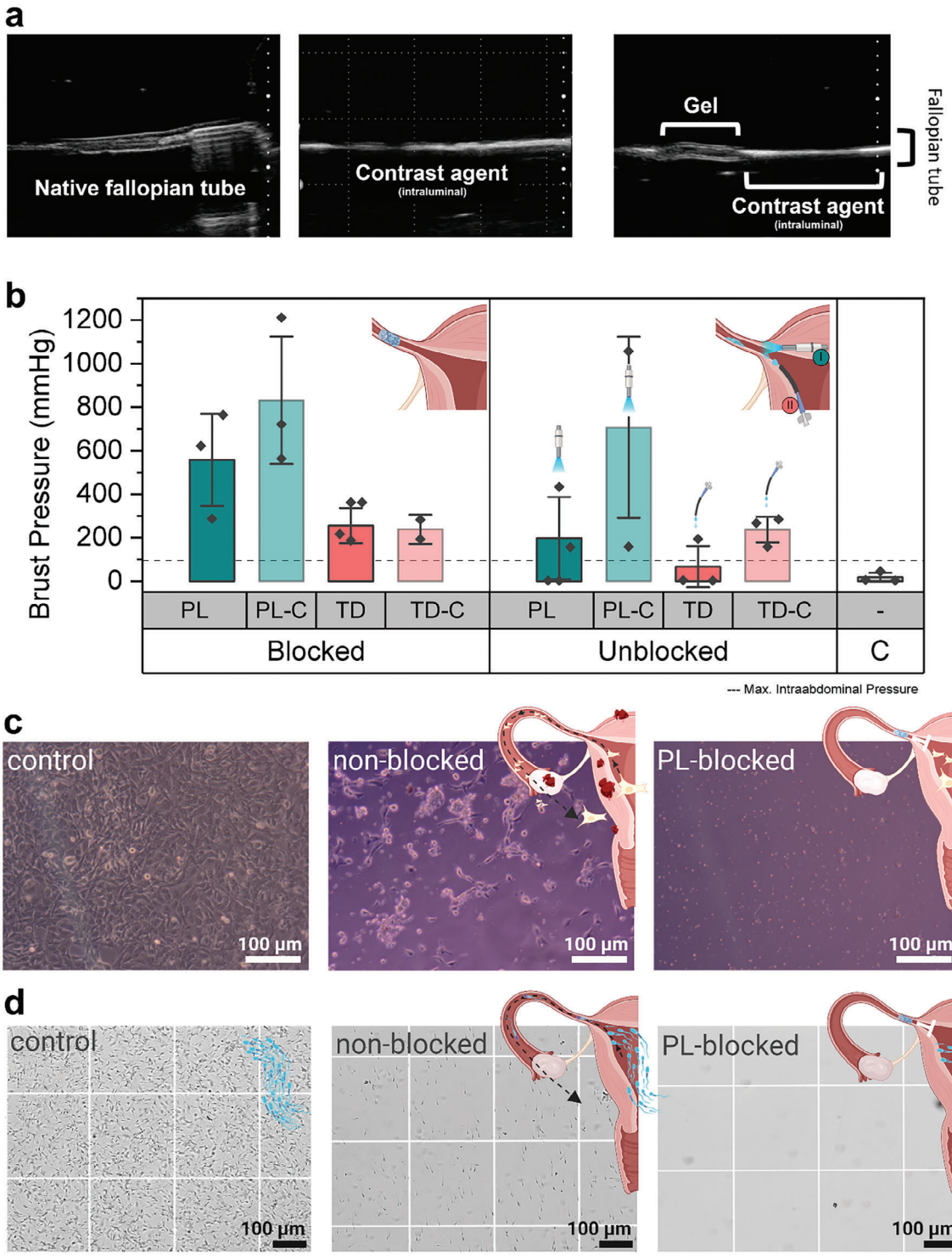

Figure 5. Effective blocking of the fallopian tube by the hydrogel implants. a) Monitoring of the effective blocking by the hydrogel implants under ultrasound using the contrast agent ExEm foam. Ultrasound image of a native fallopian tube, the unblocked fallopian tube with intraluminal ExEm foam injection, and the blocked fallopian tube with ExEm foam injection is shown. b) Burst pressure data of fallopian tube sections using simulated fallopian tube fluid. Left: After blockage with PL- and TD-Gels. Right: Fallopian tube samples after blockage and subsequent reversal using light (turquoise) or thiols (salmon) and patent fallopian tube sections (black). Maximum abdominal pressure reported in literature is indicated by the dotted line.[47] c) Endometriosis experiment. Assessment of endometrial cell passage through non-blocked and PL-blocked fallopian tubes. Control cell culture of endometrial cells (12Z) shown on the left. d) Contraception experiment. Assessment of live porcine sperm passage through non-blocked and PL-blocked fallopian tubes. All experiments were performed at least three times using independent samples (N = 3). Figures 5c and d created using Biorender.

lesions were formed. Overall, the endometrial cell blocking proof-of-concept shown here demonstrates the potential utility of the developed stimuli responsive hydrogel system as a treatment option for endometriosis. Additionally, using boar sperm from a fertilization supplier, we show that no sperm cells were able to pass through the blocked fallopian tube even after repeated cycling (Figure 5d), while a significant number of sperm cells could pass through the non-blocked tube (1.85 ± 1.36 million cells, N = 3) from initially 19.6 million live sperm cells. Taken together, these experiments show that neither sperm nor endometrial cells are able to pass through fallopian tubes blocked with hydrogel implants. How long-term blocking affects the host, and its fertility is yet to be investigated in comprehensive long term studies, however, upon completion such investigations may help shine light on potential effects on menstrual cycle.[49,50]

3. Conclusions

In this work, we proposed polymer materials with on-demand and triggerable degradation for the functional and reversible, swelling mediated, occlusion of fallopian tubes as a non-hormonal contraceptive option and/or as a mechanical option for preventing endometrial cell passage and migration. The stimuli-responsive hydrogels were found to have favorable swelling and viscoelastic properties that led to complete fallopian tube occlusion and stayed in place upon exposure to physiologically relevant pressures. In addition, the low stiffness and negligible cytotoxicity of the hydrogels indicates the system has a low risk of inducing fibrosis and causing cell damage, both of which could lead to decreased fertility and fallopian tube scarring. This was further supported by histological analysis of blocked fallopian tube sections, where no obvious damage to the fallopian tube is observed during long term in vivo experiments in a piglet model. Degradation of the hydrogels was achieved by incorporation of either a photolabile or thiol-degradable crosslink, both of which provide easily administrated and surgically relevant options for restoration of tubal patency. Perfusion of TD-Gels with GSH resulted in complete hydrogel degradation in two out of three cases and flood irradiation of PL-Gels with UV light led to a considerable decrease in burst pressure, indicating degradation. The developed hydrogels were able to be easily placed within a gynecological track model in a minimally invasive manner using ready-at-hand gynecological tools. Importantly, we show that these hydrogels have the potential to prevent the passage of endometrial cells through the fallopian tube. The functional occlusion of fallopian tubes has the potential to aid in improving our understanding of the pathogenesis of endometriosis. Further, provided that Sampson's theory of retrograde menstruation is correct, mechanical blockage of the fallopian tubes could present a novel treatment for the prevention of endometrial lesions improving the livelihood of the women suffering from endometriosis. Excitingly, we also demonstrate the successful recovery after three weeks of a hydrogel placed in vivo and observe firm contact between the hydrogel and the porcine fallopian tube and no detectable adverse effects. The materials presented here hold promising potential toward a reversible, non-hormonal contraceptive that may act as a novel alternative for the prevention and treatment for abdominal endometriosis. Clinical implementation of these materials requires more extensive in vivo investigations, particularly in human relevant models such as the commonly employed murine model.[51] Specific investigations into the compatibility and functionality of the fallopian tube integrity after hydrogel removal are keys for further translation. In particular, the ability to naturally conceive after prolonged fallopian tube implantation should be evaluated. In conclusion, the presented hydrogel materials afford a dually functional system, which constitutes a possible means of mechanically preventing endometriosis while simultaneously yielding an on-demand, reversible contraceptive tool, capable of alleviating the need for tubal ligation surgeries as a whole and improving the livelihood of women in search of new treatment options.

4. Experimental Section

Chemicals and Materials: All chemicals were purchased from Sigma-Aldrich except Formalin (ROTIHistofix 4%, ROTH) and PEGdiPDA crosslinker. The PEGdiPDA molecule was synthesized following literature protocols.[28] PEGdiPDA crosslinker and solutions containing the photolabile molecule were always stored in dark and protected from light during handling. N-(2-Hydroxyethyl) acrylamide (NHEA) monomer solution and poly(ethylene glycol) diacrylate M_n 4,000 Da (PEGdiacrylate) both contained MEHQ inhibitor and were purified by running them through a 1 cm column of basic alumina oxide (Brockmann Grade I). As PEGdiacrylate was provided in solid form, dissolution in 98% ethanol prior to purification was necessary. Later, ethanol was removed from the PEGdiacrylate filtrate using a rotary evaporator. Clean NHEA was stored at 4 °C and clean PEGdiacrylate at −20 °C. Teflon tubes used to obtain tube shaped hydrogels were purchased from VWR, 3D printing material (PETG) was purchased from Prusa. The plastic mold used for the uterus model was purchased at a local convenience store. Fresh fallopian tubes were provided by a local slaughterhouse (SBZ Schlachtbetrieb Zürich AG). Tissue samples were either used fresh (Histology) or stored at −20 °C in double sealed freezer bags. Samples were always thawed immediately before use and never refrozen. Endometrial cells (12Z) and materials used for cell culture were purchased from Abm (Applied Biological Materials Inc., USA).

Simulated Oviduct Fluid Preparation: The composition of simulated oviduct fluid was adapted from descriptions biological fluid descriptions of J. Aguilar et al.[52] and K. Ying Bonnie Ng et al.[53] More specifically a 1 L stock of simulated oviduct fluid (SOF) precursor was prepared by dissolving 5.174 g NaCl, 0.91 g KCl, 0.24 g $CaCl_2$ dihydrate, 0.207 g $MgCl_2$ hexahydrate, 1.747 g $NaSO_4$ and 2.329 g Na_2HPO_4 heptahydrate in 1L Milli-Q water. The precursor solution was stored at RT and stirred for 10 min on a stirring plate (500 rpm) before use. To prepare 0.2 L of final SOF stock, 19 mg Glucose, 192.2 mg Lactate, 3.5 mg Pyruvate and 1.4 g Albumin were dissolved in 0.2 L of SOF precursor solution. pH of final SOF was adjusted to 7.2 using NaOH or HCl. PH adjusted SOF was stored at 4 °C for no more than one week before use.

Hydrogel Preparation: Prior to preparing hydrogel master mixes (MM), a stock solution of 2 wt% N, N′-Methylenebisacrylamide (mBAA) was made by dissolution of 1 g (6.5 mmol) mBAA in 49 mL Milli-Q water. Additionally, a 2 wt% N, N'-Bis(acryloyl)cystamine (BAC) crosslinker stock was prepared by dissolving 40 mg (153.6 µmol) BAC in 980 µL Milli-Q water and 980 µL Isopropanol. Both crosslinker stocks were sonicated for 10 min and stored at 0–4 °C. Furthermore, an Ammonium Persulfate (APS) stock was prepared by dissolving 60 mg APS in 2 mL Milli-Q water. The APS stock was vortexed and stored at 0–4 °C.

To prepare the photolabile (PL) gel MM and its PEGdiacrylate control (PL-C), a 40 wt% monomer mix of 2-Acrylamido-2-methyl-1-propanesulfonic acid (AMPS) and clean, inhibitor removed, N-(2-Hydroxyethyl) acrylamide (NHEA) was made. For a 2 mL mix, 640 µL AMPS, 50 µL NHEA, 530.6 µL Milli-Q and 2.42 µL TEMED were added to a 15 mL falcon tube and vortexed for ≈1 min. Subsequently, 33.35 mg (16.7 µmol) of crosslinker (PEGdiPDA or PEGdiacrylate) was added to the mix and vortexed once more. To prepare the thiol-degradable (TD) MM, a final 25 wt% AMPS/NHEA monomer mix was made. For a 2 mL mix,

800 µL AMPS (50 wt% in Milli-Q), 100 µL NHEA (pure, inhibitor free), 1063.5 µL Milli-Q and 2.42 µL TEMED were added in a 15 mL falcon tube and vortexed for 1 min. After mixing, 34.02 µL of 2 wt% BAC stock solution was added to the MM. An mBAA control (TD-C) MM was prepared similar to the TD-MM by replacing the 34.02 µL of 2 wt% BAC with 20.15 µL 2 wt% mBAA and 13.9 µL Milli-Q water. All MM were frozen in 100 µL aliquots (−20 °C) and thawed only once, immediately prior to use.

To obtain tubes shaped gels, a long Teflon tube (ID = 1.14 mm) was cut into ≈1.5 cm long sections. A 100 µL MM aliquot was thawed, vortexed and 7.2 µL APS (30 mg mL^{-1}) was added to initiate polymerization (independent of MM type). After adding APS, gel solution was vortexed shortly (≈10 s) and injected into short Teflon tube sections (10 µL per Teflon tube section) using a 10 µL pipette. Gels were polymerized within the Teflon tubes for 15 min at 60 °C and incubated in 98% ethanol overnight at room temperature (RT). The following morning, gels were removed gently from tubing with a blunt syringe needle (20 G) and incubated in ethanol for two additional hours. Next, ethanol was removed, and gels were dried in a vacuum oven (10 mbar, 40 °C) over night. Dry gels were stored in tightly closed glass vials at room temperature.

Swelling Experiments: For swelling experiments, samples of all four gel types (PL, PL-C, TD and TD-C) were prepared in triplicates. Gels were made similar to the tube-shaped gels described above; However, instead of polymerizing 10 µL of gel solution within Teflon tubes, 50 µL droplets were polymerized on Teflon plates. Also, prior to the drying process, each gel was transferred into a separate glass vial. The weight of each vial was measured with and without a gel and the "as-prepared" initial hydrogel mass M_i was calculated.

$$M_i = M_{Vial+Gel} - M_{Vial} \quad (1)$$

After the drying process, 5 mL of SOF was added to each vial and the samples were incubated at 37 °C on a lab shaker (IKAKS 130 basic, 80 rpm). At different timepoints (0, 30 min, 1 h, 2 h, 4 h, 18 h, 24 h), remaining SOF was removed with a 20 G Sterican Syringe Needle and residual hydrogel mass was calculated as described above. Relative swelling ratios were calculated as follows:

$$S_R = \frac{M_{Swollen} - M_i}{M_i} \quad (2)$$

It is notable that for timepoint 0 min the $M_{swollen}$ corresponds to the mass of the lyophilized gel with M_i being the mass of the gel directly after radical polymerization.

SEM and FTIR: Fourier-transform infrared spectra of as-prepared hydrogel samples were measured using a Varian 640-IR spectrometer equipped with diamond attenuated total reflectance (ATR) optic. Scanning electron microscopy (SEM) images were taken using an Axia Chemi SEM (Thermo Fisher) at an accelerating voltage of 10 kV. Imaging was performed on uncoated, as-applied (d = 1.14 mm), hydrogel samples at low vacuum mode. Hydrogel samples were cut with a scalpel and mounted on a 90° angled SEM pin stub using carbon tape.

Gel Stability in Human Peritoneal Fluid: Human peritoneal fluid was obtained during laparoscopic surgery on a woman in her childbearing years at Kantonsspital St. Gallen (KSSG). A laparoscopic suction device was used to remove ≈14 mL of peritoneal fluid from the posterior cul-de-sac. Collected fluid was stored at −20 °C for less than one month. A consent form was signed by the patient. To test gel stability in human peritoneal fluid, 2 mm long, as-applied hydrogels were immersed in 2 mL of human peritoneal fluid. Gels were swollen for 24 h at 37 °C, under slight shaking (80 rpm). After 24 h, remaining fluid was removed carefully, and gel stability was assessed qualitatively.

Rheology: Rheological measurements were used to characterize viscoelastic properties and degradation kinetics of swollen hydrogels. Three sets of experiments were conducted. First, polymerization times and storage moduli of as-prepared hydrogels were assessed by in situ polymerization of PL and TD Gel master mixes (T = 60 °C). Second, storage moduli of swollen gels (PL and TD) and porcine FT tissue samples were measured. To fit the rheometer geometry (d = 8 mm, sandblasted), oviduct samples were cut longitudinally, flattened, and punched through with an 8 mm disposable biopsy punch (KAIMedical). Gel samples were polymerized in (3 mm and 4 mm) silicone molds (McMaster-CARR Duro Red), dried, swollen and embedded in 4 wt% Agarose in PBS. Embedded gels were cut into 300 µm slices using a Compresstome (VF-310-0Z, Precisionary). Hydrogel and tissue samples were placed on the rheometer (Anton Paar MCR 502) and kept wet with PBS during measurements. To determine degradation kinetics of PL-Gels, 300 µm PL-Gel samples were irradiated with UV light (λ = 365 nm, I_0 = 9.6 mW cm^{-2}) using a liquid light quid coupled to an LED light source (ThorLabs DC4104 – 4-Channel LED Driver). Photodegradation first-order rate constant was calculated by normalizing storage modulus (G') data and performing linear regression on a data subset ($0.5 \leq G' / G'_0 \leq 1$).[40,54] FT rheological experiments were conducted in triplicates. All other measurements were done once for each experimental group.

Surgical Application Model—Uterus Model: To create a negative model of the uterine cavity and the fallopian tubes, a 22 wt% acrylamide/gelatine mix was polymerized around a 3D printed model of the uterus. More precisely, an anatomical model of the human uterus (with fallopian tubes) was downloaded from sketchfab.com and modified using Tinkercad. Fallopian tubes were elongated, and the entire model was scaled to fit the size of a human uterine cavity. The modified model, together with a 10 cm long cylinder (d = 2.5 cm) was printed with a PrusaMini 3D Printer using Prusament PETG filament. The cylinder was attached to the cervical part of the model and the inner wall of a 2 L plastic mold. (Figure S2a, Supporting Information). Hot glue was allowed to harden overnight.

To prepare the 22 wt% acrylamide/gelatine mix, 40 g of Gelatine powder was dissolved in 1.56 L Milli-Q water by repetitive shaking and heating in a 60 °C preheated oven. Next, 400 g of Acrylamide was added to the mix under continuous stirring and heating (500 rpm, 60 °C). Once all gelatine and acrylamide were dissolved, 21.6 mL of 2 wt% mBAA Stock and 3.2 mL TEMED were added to the mix. Also, a 50 mL stock of Ammonium Persulfate (APS) solution was prepared by dissolving 3 g of APS in 50 mL Milli-Q.

The Tupperware was filled layer by layer with 200 mL of Aam/Gelatine mix. For each layer, 4.48 mL APS (60 mg mL^{-1}) was added drop by drop to 200 mL gel mix. After initiating polymerization, the gel was allowed to polymerize and cool down (at RT) for at least 30 min to avoid melting of the plastic mold and the 3D model. Final Aam/Gelatine model (Figure S2b, Supporting Information) was stored at 0–4 °C.

The uterus model was examined by a gynaecologist at the medical training center in St. Gallen (Ostschweizer Schulungs- und Trainingszentrum, KSSG). The Aam/Gelatine model was placed in a water bath and a hysteroscope (Karl Storz, 26120BA, 26153BIK, 26153BOK) was inserted through the cervix (Figure S3a, Supporting Information) into the uterine cavity of the model. Images and videos were taken using the Tele Pack X LED Monitor (Karl Storz, TP 100). Gel insertion was simulated using a urinary catheter (Cook Medical, G14430) and a small Teflon tube (OD = 0.8 mm). Unfortunately, gels produced in Teflon tubes (ID = 1.14 mm) were too large for the paediatric catheter and actual gels could not be placed using the hysteroscope.

Surgical Application Model—Ultrasound: Ultrasound images of our model were taken using a linear scanner (Clarius, Model L7HD) coupled to a smartphone using the Clarius Ultrasound App. Imaging was performed on the Aam/Gelatine model with patent and occluded fallopian tubes while immersed in a water bath.

Gel Cytotoxicity and In Vivo Cytocompatibility: Perfused cell medium experiments were performed as described by Anthis et al.[32] using CytoTox 96 Non-Radioactive Cytotoxicity Assay (Promega). Normal Human Dermal Fibroblasts (NHDFs), a non-cancerous human skin fibroblast cell line, was cultured under standard culture conditions at 37 °C with (5%) CO2. Dulbecco's Modified Eagle's Medium – high glucose (DMEM), supplemented with 10% Fetal calf serum, 1% L-Glutamine 1% Penicillin-Streptomycin-Neomycin Solution was used as full growth medium. Dried PL- and TD-Gel samples (20 µL) were swollen in 2 mL of full growth medium within (15 mL) falcon tubes at 37 °C. After 24 h, media was removed (1st Extraction) and fresh media (2 mL) was added for a second incubation period of 24 h (2nd Extraction). A second set of samples, used

to investigate cytotoxicity of degradation products, were swollen in 2 mL Dulbecco's Phosphate-buffered saline (PBS, D8537). After 24 h incubation, PBS was not removed, and swollen gels were degraded within the falcon tubes (Deg.). PL-Gel samples were irradiated with UV light (λ = 365 nm) for 30 min and TD-Gel samples were incubated at 80 °C for 24 h to break disulfide bonds. Extracted media and PBS of each experimental group was collected in (15 mL) falcon tubes, immediately frozen in liquid nitrogen and stored at −20 °C for less than one week.

4000 NHDF cells were seeded in (100 µL) full growth medium and allowed to attach for at least 24 h in a 96 well plate. 100 µL of extracted cell media or PBS (containing gel degradation products) was added to the cells (total volume 200 µL). Also, 100 µL of fresh PBS (negative control) and 100 µL full growth media containing 10 µL of 10X Lysis Solution (positive control) were added to the cells. The plate was then incubated under standard culture conditions for 24 h. Cell viability was assessed through an LDH cytotoxicity assay using CytoTox 96 Cytotoxicity assay (#G1780, Promega, Dübendorf, Switzerland). Three independent experiments were executed per experimental group.

An in vivo proof of feasibility study was performed using a piglet model. The study was conducted under the supervision of the Commission of Work with Experimental Animals at the Medical Faculty of Pilsen, Charles University, certified under project ID: MSMT-21623/2023-2, and was monitored by the Ministry of Agriculture of the Czech Republic. All procedures strictly adhered to the laws of the Czech Republic, which align with the regulations of the European Union. We conducted the proof-of-concept experiments in two pigs, and we present data from one representative pig. The subjects were healthy female Prestice black-pied pigs, 14 weeks old, weighing 30–40 kg, and they received intramuscular premedication with ketamine (Narkamon 100 mg mL−1, BioVeta a.s. Ivanovice na Hané, Czech Republic) and azaperone (Stresnil 40 mg mL−1, Elanco AH, Prague, Czech Republic). General anesthesia was initiated and maintained using propofol MCT/LCT (Propofol 2% MCT/LCT Fresenius Medical Care a.s.). Nalbuphin (Nalbuphin, Torrex Chiesi CZ s.r.o., Prague, Czech Republic) was used for analgesia. Midline incision laparotomy was performed to enter the abdominal cavity. The hydrogel implants were surgically implanted into the oviducts of two female piglets. The abdominal wall was closed by polydioxanone monofilament 1 suture (PDS II, 1, Ethicon, Johnson & Johnson, NJ) and the pigs were monitored closely for 21 days with ad libitium access to food and water. After 21 days, the piglets were anaesthetized and then immediately sacrificed using a cardioplegic solution and tissue samples were harvested and fixed in formalin. The entirety of the experiment was documented via photography.

Burst Pressure—Sample Preparation: Frozen fallopian tubes were thawed for 30 min in lukewarm water before use. First, the fallopian tubes and the uterotubal junction were identified. Soft tissue surrounding the fallopian tubes was dissected using surgical scissors (Figure S3a, Supporting Information). Residual parts of the uterine horn were removed together with the uterotubal junction and the proximal part (Isthmus) of the oviduct was cut into three (≈3 cm long) sections (s1, s2, s3). Blunt (22 G) syringe needles were carefully inserted into the tube sections and placed into a (15 mL) falcon tube filled with 5 mL of Formalin (ROTIHistofix 4%, ROTH). Tissue sections were incubated in Formalin at room temperature (RT) for at least 24 h. After fixation, oviduct sections were removed, washed with PBS, and transferred into a different (15 mL) falcon tube containing 10 mL of PBS. Syringe needles were removed after 24 h incubation in PBS, oviduct sections were flushed with PBS and transferred in falcon tubes containing fresh (10 mL) PBS. After an additional incubation period of 4 h, fallopian tube sections were flushed again with PBS and as-applied hydrogels were inserted to block the tubes. More precisely, tube shaped gel samples were cut into 2 mm long sections with a scalpel and carefully inserted into ≈2 cm long Teflon tubes (Figure S3b, Supporting Information). The Teflon tube sections were then inserted ≈1 cm deep into the fallopian tube and the gel was pushed into the FT using a (22 G) blunt syringe needle (Figure S3b, Supporting Information). For each gel type (PL, PL-C, TD, and PT-C), tissue samples from the same fallopian tube were used (e.g., s1, s2, and s3 of FT 1 were all blocked with PL gels). After gel insertion, oviduct sections were incubated (at 37 °C) for 24 h, in 10 mL fresh SOF.

Burst Pressure—Light Degradation: A 600 µm medical fiber (NA = 0.37, SMA905 / FC connector, Wuhan Medfibers Technology) was coupled to an LED light source (FC1-LED-365A, Prizmatix). Output power of the LED light source could be manually controlled. Peak light intensities of ≈40 mW cm^{-2} were measured at the tip of the medical fiber using a laser power & energy meter (SOLO PE, S/N 154 961). The medical fiber coupled to the LED light source was used at maximal power to degrade photolabile hydrogels within FT segments (Figure S5a, Supporting Information). More specifically, the tip of the medical fiber (protective silicone removed) was inserted into a fixed and blocked FT section until slight resistance of the gel was felt. The LED light source was turned on and set to its maximal power. Gels were irradiated for a total of 30 min. After 10 min, 20 min and 25 min irradiation, the fiber was removed, and the FT section was flushed very carefully with (2 mL) SOF using a small (23 G) blunt syringe needle. Following light irradiation, samples were incubated again in SOF for 24 h at 37 °C. FT sections blocked with PL and PL-C gels and irradiated with light were referred to as PL and PL-C unblocked FT samples.

Burst Pressure—Thiol Degradation: To degrade TD-Gels within fallopian tube segments, a closed loop perfusion system was built (Figure S5b, Supporting Information). More precisely, a 3D printed hollow cylinder (PETG, ID = 45 mm, OD = 55 mm) was connected to a submersible mini water pump (DC 3–6 V, flow rate ≈800 mL m^{-1}n) using silicone tubing (ID = 3 mm). The cylinder was divided into four compartments and the cylinder bottom (≈1 cm) was filled with steel wool to avoid blockage of the system. Fixed FT segments blocked with TD- or TD-C-Gels were placed into each compartment. The perfusion system was filled with 100 mL 0.2 M Glutathione (GSH) solution (preheated to 37 °C). The GSH solution was prepared by dissolving 6.146 g L-Glutathione reduced in 50 mL PBS, adjusting the pH to 7.2 using (≈25 mL) 1 M NaOH and adding additional PBS until a total volume of 100 mL was reached. The entire system was placed into a water bath (37 °C) and the pump was turned on. Following 30 min of continuous perfusion with GSH solution, samples were removed and incubated in fresh SOF for 24 h at 37 °C. FT sections blocked with TD and TD-C gels perfused with GSH solution were referred to as TD and TD-C unblocked FT samples.

Burst Pressure—Burst Pressure Measurements: The 50 mL syringe was filled with 10–20 mL fresh SOF and mounted on the syringe pump. The pumps flow rate was set to 1 mL m^{-1}n and the silicone tubing system was attached to the syringe. The tubing was filled with SOF by tuning on the pump for ≈30 s. Once all air was removed from the system, the three-way valve was closed. Next, the fallopian tube segments were attached to the (20 G) blunt syringe needle with a haemostat (Figure S4, Supporting Information). For continuous pressure measurement, the sensor was connected to a computer and pressure values were monitored using a Sensor Evaluation Kit (Honeywell, SEK002 Version 1.1). Before attaching the sensor to the silicone tubing, pressure input was set to zero (Auto Zero). Once the sensor was attached, the pressure recording was started, and the syringe pump was turned on. The measurement was stopped once a significant drop in pressure was detected or no change in pressure occurred within 20 s. Three independent burst pressure measurements were carried out per experimental group.

Histology: Fresh fallopian tubes (FT) from SBZ Schlachtbetrieb Zrich AG were collected early in the morning, transported on ice, and dissected within 1 h. Fresh tubes were blocked with hydrogel particles, incubated in SOF for 4 h and fixed in 4% Formalin right after incubation to preserve the tissue. Fixed tissue samples were sent to Sophilstolab AG, Muttenz, Switzerland for histological analysis. Samples were embedded in paraffin blocks, sliced, and stained using H&E. Images were scanned and analyzed using ImageScope.

Endometriosis Model—Cell Culture: The well characterized, lesion-forming human endometrial cell line EE12Z[31,55] was purchased from Abm (Applied Biological Materials Inc., USA) after signing a Material Transfer Agreement (MTA). The cell line was maintained according to propagation requirements of manufacturer. Prigrow III medium (Abm TM003) was supplemented with 10% fetal bovine serum (Invitrogen F9665) and 1% of Penicillin/Streptomycin Solution (Gibco P4458) to obtain a complete growth medium. PriCoat T25 Flasks (abm, G299) were

used for cell culture and media was changed every 2–3 days. Cells were cultured in a humidified incubator (37 °C and 5% CO2) using PriCoatTM T25 Flasks (abm, G299) and cell culture medium was changed every 2–3 days.

Endometriosis Model—Flow System: To mimic retrograde menstruation through the fallopian tubes or insemination, a flow system similar to the burst pressure system was used (Figure S7a, Supporting Information). A 10 mL syringe (Omnifix B Braun) was mounted on a programmable syringe pump (NE-1000, KF Technology). A single silicone tube (ID = 3 mm) was used to connect the 10 mL syringe with a blunt (20 G) syringe needle. The flow rate and dispensing volume of the programmable syringe pump were adjusted to simulate physiological pressures (bursts of up to 50 mmHg) occurring in the fallopian tube.[48] More precisely, the pump was programmed to enter a continuous loop of dispensing (+0.15 mL) and filling (−0.15 mL) cycles at a rate of 1 mL m^{-1}n with 10 s breaks in between. Evaluation of volumes and flow rates needed to reach physiological pressures were done using the tubing system and the pressure sensor of the burst pressure set-up (Figure S7b, Supporting Information).

Endometriosis Model—Endometriosis Model Experiments: Three different sets of experiments were conducted (Figure S7c, Supporting Information). First, cytocompatibility of the designed flow system was investigated in relevant timeframes. Second, passage of endometrial cells through non-blocked fallopian tube sections were assessed. Third, functional blockage of stimuli degradable hydrogels was evaluated.

To investigate cytocompatibility, the system was filled with 4 mL of cell medium containing 1 million endometrial cells (12Z). A haemostat was used to attach a short silicone tube (ID = 1 mm) to the syringe needle of the flow system. To simulate blockage, a second haemostat was used to clamp the short silicone tube. Endometrial cells were subjected to 30 min of continuous dispensing and filling cycles (freq. ≈1.6 cycles/min). Afterward, cells and media were collected and an LDH cytotoxicity assay was performed immediately (data not shown) and after 24 h of subsequent cell culture. Assessment of endometrial cell passage was done similarly with the difference that either a non-blocked (n = 3) or a PL blocked (n = 3) fallopian tube segment was attached to the needle. Additionally, the fallopian tube segment was immersed in 4 mL fresh cell media ("Collection Media"). After the experiment, the collection media was centrifuged at 200 g for 2 min and 2 mL of supernatant was carefully removed. Next, cells were carefully resuspended in the remaining 2 mL of collection media and the number of cells which passed though the tubes were counted using a Neubaur chamber and trypan blue staining. Remaining collection media was cultured in twenty four well plates at a cell density of 40 000 cells/500 µL and incubated for 24 h. A static control was used as reference. Microscopy images were taken the next day. The experiment was repeated also for durations of 4 h of continuous dispensing and filling cycles, this time using fixed cells (to avoid cell lysis).

Sperm Passage Experiments: For the sperm passage experiments, fresh (live) boar semen was obtained within 2 h of collection from SUISAG, Zurich, Switzerland. The sperm were counted using a hemocytometer and diluted to 19.6 million cells per mL, similar to the concentration utilized for artificial insemination, using semen extender. The diluted semen sample was loaded into a syringe (4 mL sample volume) and perfusion experiments were conducted for 30 min as de-scribed above. After 30 min, the collection media was centrifuged at 200 g for 2 min, the supernatant removed, and the sperm carefully resuspended in 1 mL of semen extender. The number of sperm that passed through the tubes were counted using a hemocytometer and imaged (Zeiss Imager.M2m). Experiments were executed in triplicates (N = 3).

Statistical Analysis: No data pre-processing was applied. All measurements were carried out in triplicate, unless stated otherwise. Data is plotted as mean ± standard deviation.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

A.H.C. Anthis, S. Kilchenmann, T. Rduch and I.K. Herrmann declare inventorship on a patent application submitted by ETH Zurich and Empa (EP24167147). All other authors declare no conflict of interest.

Author Contributions

A.H.C.A. and S.K. contributed equally to this work. A.H.C.A. and I.K.H. conceived the project in discussion with T.R.; A.H.C.A. developed the polymer formulations; S.K. performed the experimental work; M.M. helped with cell culture studies; M.W. and P.L.V. synthesized the crosslinker and performed rheology measurements; P.L.V. performed sperm cell perfusion experiments; C.M. helped establish the ex vivo fallopian tube model; O.C. helped with the light setup and the fiber coupling; M.W.T. supervised the crosslinker synthesis and rheology measurements; J.R and V.L. performed and supervised the in vivo biocompatibility study; T.R. helped with hysteroscopy; A.H.C.A., S.K., and I.K.H. wrote the first draft; I.K.H. led the overall project and acquired funding; All authors contributed to the analysis, interpretation, and discussion of the results and edited the manuscript.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords

implant, non-hormonal treatment, on demand, retrograde menstruation, women's health, Sampson's theory

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