Original Article

In Vivo Evaluation of the Efficacy and Safety of a Novel Degradable Polymeric Film for the Prevention of Intrauterine Adhesions

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ABSTRACT

Study Objective: To study the safety of a degradable polymeric film (DPF) and its efficacy on reducing the risk of intrauterine-adhesion (IUA) formation in a rat model.

Design: A series of case-control studies relying on random allocation, where feasible.

Setting: University and good practice animal laboratories.

Animals: The animal models comprised female and male Oncins France Strain A and female Wistar rats.

Intervention(s) and Measurements: The Oncins France Strain A rats were used for in vivo evaluation of the impact of the DPF on endometrial thickness and its effect on fertility. For in vivo evaluation of the biologic response, 40 Wistar rats were randomly allocated to intervention and control groups, with matched sampling time after surgery. Finally, for the in vivo evaluation of the DPF’s efficacy on IUA prevention, a total of 24 Wistar rats were divided into 3 groups: 1 treated with the DPF, 1 treated with hyaluronic acid gel, and a sham group.

Main Results: The DPF did not have a significant impact on endometrial thickness, and there were no significant differences in the number of conceived or prematurely terminated pregnancies, confirming its noninferiority to no treatment. The DPF did not induce irritation at 5 days and 28 days. Finally, the DPF significantly reduced the likelihood of complete IUA formation compared with hyaluronic acid gel− and sham-implanted animals, where only 27% of the animals had their uterine cavity obliterated compared with 80% and 100%, respectively.

Conclusion: The DPF is a safe film that is effective in preventing IUA formation after intrauterine curettage in rats. Journal of Minimally Invasive Gynecology (2020) 00, 1−7. © 2020 AAGL. All rights reserved.

Keywords: Adhesions; Animal model; Intrauterine; Medical device; Synechiae

Intrauterine adhesions (IUAs) result from an endometrial trauma leading to a partial or complete obliteration of the uterine cavity [1,2]. A meta-analysis of 10 randomized controlled trials involving 912 women calculated an IUA prevalence after miscarriage of 19.1% (95% confidence interval [CI], 12.8%−27.5%) [3]. IUAs can also occur after intrauterine surgical interventions for the management of uterine leiomyomas, septa, and polyps. A diagnosis of
IUAs can be suspected when pain or abnormal bleeding occurs after surgical or obstetric intrauterine trauma [4]. Although iatrogenic trauma is established as 1 of the main precipitating factors, the exact underlying pathophysiology of IUA formation is less well known. IUAs can lead to primary or secondary subfertility, early or late miscarriage, and increase the risk for several obstetric complications, including abnormal placentation, postpartum hysterectomy, prematurity, and cesarean section [3,5–8].

Hysteroscopy remains the mainstay for IUA diagnosis and treatment; nevertheless, it carries the risk of IUA recurrence [9–11]. Several antiadhesive strategies, including hormonal therapy, intrauterine devices, intrauterine balloons, and barrier gels, have been evaluated with controversial results [12,13]. Although there is evidence that barrier gels can reduce the incidence and severity of de novo IUAs after operative hysteroscopy [13], there is limited evidence relating to their effectiveness on improving pregnancy rates [14]. We previously described the efficacy of a degradable polymeric film (DPF) in a rat model on postoperative abdominal adhesion [15,16]. The aim of the present study was to assess the safety of the DPF, its efficacy on reducing the risk of IUA formation, and its impact on fertility in a rat model.

Materials and Methods

Polymer Synthesis and Shaping

The DPF was synthesized at the biopolymer laboratory at the University of Montpellier, France, and previously described by Leprince et al [16]. For the evaluation of endometrial thickness and fertility impact, the DPF was shaped by hot extrusion with a powder filament extruder (Noztek, Shoreham-by-Sea, United Kingdom) to obtain a polymer filament with a diameter of 1.5-mm. For the evaluation of biologic response and IUA prevention, the DPF was synthesized at PolymerExpert (Pessac, France) and shaped by hot pressure to obtain a film (10 mg; length 1.3 ± 0.1 cm). The copolymer was sterilized by irradiation between 27.7 kGy and 36.6 kGy by IoniSos (Chaussenil, France). The DPF is now manufactured under the brand name of “Womed Leaf” (Womed SAS, Montpellier, France).

Animal Preparation

The evaluation of endometrial thickness and fertility was conducted at the Experimental Department of the University of Montpellier (Montpellier, France) on Oncins France Strain A (OFA) female rats (7–8 weeks old) weighing between 250 g and 275 g. OFA male rats weighing between 275 g and 300 g (purchased from Earl Cegav SSC, St. Mars d’Egrenne, France) were also required for the fertility experiment. The evaluation of biologic response and IUA prevention was conducted at Phycher Bio Développement (Pessac, France), under good laboratory practice, on female Wistar rats (specific pathogen free caw; 9–10 weeks old), supplied by Janvier Labs (Le Genest-Saint-Ise, Mayenne, France). The animal strain used depended on availability at the time of conducting the experiment. All animal investigations were approved by the ethics committee of the French Ministry of Education and Research (contract number 02367.01, task order 1065, and ecart number 76) and carried out in accordance with European Union Directive 2010/63/EU for animal experiments. All efforts were made to minimize animal suffering or distress and to use the minimum number of animals necessary to produce reliable scientific data. All the animals were in quarantine for 1 week before treatment. They were placed individually in an air-conditioned animal-holding facility (temperature 22˚C ± 3˚C; humidity 30%–70%) with free access to food safe means safe food for this type of animal and water. They were examined, weighed, and their litter changed daily, respecting the guidelines of good practice and animal welfare.

The OFA rats were anesthetized by intravenous perfusion of ketamine (50 mg/kg) and acepromazine (0.5 mg/kg). Anesthesia for the Wistar rats was induced and maintained by inhalation of an oxygen–isoflurane mixture (IsoFlo; Zoetis Inc., Kalamazoo, MI). Before surgery, the OFA and Wistar female rats were weighed and injected with buprenorphine (Buprecare at 0.05 mg/kg) and meloxicam (Meloxidolor at 0.5 mg/kg) subcutaneously. After anesthesia, the animals were shaved in the abdominal region, disinfected with polyviode iodine, and wiped with 70% isopropyl alcohol, after which a sterile drape was placed at the operative site. One mL subcutaneous lidocaine 0.1% was injected before the cutaneous incision. An abdominal vertical skin incision was performed, followed by dissection with scissors of the subcutaneous and muscle fascia tissues. The peritoneum was opened with scissors, allowing access to the abdominal cavity to rule out organic disease of the reproductive system (uterus, oviducts, and ovaries). The uterus was exteriorized for the procedure. For both types of animals, buprenorphine (0.02 mg/kg) was systemically administered twice a day for 3 days. At the end of each protocol or in case of reaching the tolerance-limit point, the animals were euthanized by lethal injection of sodium pentobarbital after anesthesia by ketamine to limit the pain. No animals were reused for other experiments.

In Vivo Evaluation of the Impact of the DPF on Endometrial Thickness

The objective was to evaluate the impact of the DPF on the rat’s uterine horn endometrial thickness, as measured histologically. This study was performed on 7 OFA female rats. After animal preparation, the right horn’s extremity was opened and the DPF inserted (Supplemental Fig. 1A). No DPF was placed in the left horn (control). The horns’ extremities were ligated to limit film expulsion (Supplemental Fig. 1B). The operative and postoperative complications were recorded. Histologic
hematoxylin−eosin embedded in paraffin wax. The samples were analyzed after formaldehyde 3.5% and phosphate-buffered saline), and in rats. The horns were split longitudinally, fixed in formalin−alcohol−acetic acid medium, dehydrated, and then embedded in paraffin wax. Sections of approximately 4 μm were stained with hematoxylin−eosin before assessment, using the NF-ISO 1993-6 grading system (available on request). The irritation score was calculated on the basis of the mean of the sum of tissue damage (necrosis) and cellular inflammatory parameter scores weighted with a factor of 2 plus the repair phase of inflammation (fibrosis and neovascularization) and fatty infiltrate parameter scores. The irritation index, reflecting the intensity of the inflammatory process and the local tissue effects, was determined by subtracting the irritation score of controls from the score of DPF-implanted animals within each of the paired groups. An irritation index of 0 to 2.9, 3.0 to 8.9, 9.0 to 15.0, and >15 was considered non-, slightly, moderately, or severely irritant, respectively. Distant organs (e.g., stomach, intestine, rectum, liver, spleen, inguinal nodes, heart, aorta, pancreas, kidneys, and vagina) were macroscopically and microscopically evaluated at 28 days.

In Vivo Evaluation of the Impact of the DPF on Fertility

The reproductive outcome was evaluated in a prospective randomized study of 20 OFA female rats. Randomization was used to determine which horn was to be implanted. The selected horn was incised, the DPF was inserted, and the horn was sutured; the other horn was used as control. The main end point was the number of pregnancies per horn. The purpose of the test was to demonstrate the noninferiority of the DPF-treated horns compared with those of the controls. The female rats were confined in individual cages for 15 days to allow for abdominal healing and DPF degradation as demonstrated in our previous study [16]. On day 15, they were mated with 5 OFA males to mitigate potential bias caused by variation in sperm parameters. Fifteen days after mating, manual palpation of the abdomen and abdominal ultrasound were used to confirm the pregnancies. The animals were then euthanized and the number of fetuses counted in each horn.

In Vivo Evaluation of the Biologic Response to the DPF

In total, 40 Wistar female rats contributed to this experiment. The animals were randomly allocated to 1 of 6 groups arranged in 3 pairs: groups 1 and 2 (5 intervention animals and 5 controls, sampled 24 hours after surgery); groups 3 and 4 (5 intervention animals and 5 controls, sampled 5 days after surgery); and groups 5 and 6 (10 intervention animals with left horn implantation and 10 controls with left horn incision only, sampled 28 days after surgery). A 4-cm-long longitudinal midline abdominal incision was made. The uterine horn was exteriorized from the abdominal cavity and incised over its entire length. Depending on the group allocation, the endometrial lining was abraded by scratching with a scalpel blade to remove the epithelial layer ± implanted agent [17]. The uterine horn was then ligated close to the uterine junction with the other horn and 2 mm to 3 mm under the abraded area without cutting off blood circulation to keep the DPF and HA gel in contact with the uterine horn. After 7 days, the animals were euthanized and the uterine horns preserved in an alcohol−formalin−acetic acid medium, dehydrated, and then embedded in paraffin wax. Caudal, medial, and cervical sections were taken from each horn and stained with hematoxylin−eosin. The presence of intrauterine adhesions was evaluated for each uterine horn. The incidence of IUAs was defined as the presence of complete adhesion in at least 1 of the 3 sections of the horn.

Statistical Analysis

Before the reproduction study, a power analysis, conducted with a significance level of 5% on the basis of the
variance from a statistical 2-sample $t$ test, resulted in a power of 80%. The mean endometrial thickness, calculated from 3 measurements per horn, were compared in the intervention and control arms using the Mann–Whitney U test. The mean number of pregnancies per horn were compared in the intervention and control arms using a paired $t$ test. Fertility noninferiority was based on the difference in the average number of ongoing and prematurely terminated pregnancies in the DPF and control groups. The a priori thresholds for noninferiority were $-1$ and $0.5$ for the total number of ongoing pregnancies and the number of prematurely terminated pregnancies, respectively. The lower and upper bounds of the CIs for the difference in the average number of ongoing and terminated pregnancies, respectively, were compared with these thresholds to assess non-inferiority. The efficacy of the DPF for IUA prevention was assessed using the Fisher exact test because the comparison of proportions of the existence of adhesions was on a small sample of $<30$.

Results

**In Vivo Evaluation of the Impact of the DPF on EndometrialThickness**

The endometrial thickness was noted to be thinner on day 1 in the DPF group than in the control group. However, the difference in thickness at all the implantation time periods (1, 5, and 12 days) was not significantly different between the groups (Table 1).

<table>
<thead>
<tr>
<th>Day of analysis</th>
<th>DPF group (n = 7)</th>
<th>Control group (n = 7)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>337.3 ± 118.9</td>
<td>693 ± 371.8</td>
<td>.09</td>
</tr>
<tr>
<td>Day 5</td>
<td>897 ± 441</td>
<td>927.8 ± 226</td>
<td>.11</td>
</tr>
<tr>
<td>Day 12</td>
<td>647.3 ± 147.9</td>
<td>777 ± 406.5</td>
<td>.2</td>
</tr>
</tbody>
</table>

DPF = degradable polymeric film.

**In Vivo Evaluation of the Impact of the DPF on Fertility**

A total of 144 and 131 pregnancies were clinically observed in the DPF and control groups, respectively, and this difference was not statistically significant. The average of total pregnancies identified per horn was $7.2$ ($±2.56$) for the DPF group compared with $6.5$ ($±2.81$) in the controls, a difference of $0.65$ ($p = .38$), whereas there were $9$ prematurely terminated pregnancies in the polymer group and $18$ in the control group ($p = .09$). The mean difference in ongoing pregnancy between the DPF and control groups was $1.1$ (95% CI, $0.089–\infty$), whereas the mean difference in prematurely terminated pregnancies was $-0.45$ (95% CI, $-\infty$ to $-0.007$). These results support DPF noninferiority because the lower bound of the CI for the mean difference in ongoing pregnancy was greater than, and the upper bound of the CI for the mean difference in prematurely terminated pregnancies was lower than, the preset noninferiority threshold of $-1$ and $0.5$, respectively.

**In Vivo Evaluation of the Biologic Response to the DPF**

One animal in the control group was found dead on day 7, and missing small intestine and cecum were the only macroscopic findings recorded at necropsy. Apart from this, we did not observe any unexpected clinical signs in either the DPF or control groups. Macroscopic and microscopic examination of the animals at 28 days did not reveal changes in the main organs of the treated or control groups. There were no macroscopic anomalies seen in the inguinal lymph nodes draining the implantation sites. Fig. 1 illustrates an example of the histopathologic examinations of the uterine horns at 24 hours, 5 days, and 28 days after implantation in both the DPF and control groups. Examinations showed that 24 hours after implantation, the DPF induced a minimal to mild inflammatory process within the endometrium in 4 out of 5 animals. This was characterized by an infiltration of the polymorphonuclear cells and/or macrophages and was associated with the flattening of the epithelial cells, focal or localized extensive erosion, and a luminal exudate in the DPF group when compared with the control uterine horns. At 5 days, the induced inflammatory process was still observed within the endometrium but at a lower incidence (3 out of 5 animals) and severity when compared with the 24-hour period. At 28 days, the macroscopic examination of the implantation sites showed that the segment of uterine horns between the ligature made during the implantation procedure and the ovaries were hypertrophied and fluid-filled in 8 out of 10 and 8 out of 9 females from the DPF and control groups, respectively. These changes were bilateral in 7 of the 8 affected animals in each group. Histopathologic examinations showed that luminal dilatation, recorded in 5 out of 10 and 8 out of 9 females of the DPF and control groups, respectively, was the histologic correlate of the hypertrophied uterine horns observed at necropsy. Hence, these findings were deemed secondary to the implantation procedure. No mortality attributable to the DPF occurred during the study. Table 2 displays the biologic response of uterine tissues in the DPF-treated and control groups. The DPF was classified as slightly irritant (irritation index 3.6) at the end of the 24-hour period and nonirritant at the end of the 5-day period (irritation index 1.6) and 28-day period (irritation index 0) (raw data available on request).

**In Vivo Evaluation of DPF Efficacy on IUA Prevention**

In the sham group, 100% (15 out of 15 horns) had at least 1 complete IUA (Fig. 2A) in the 3 regions of the
uterine horn. When comparing the DPF- and HA-treated groups, 26.7% (4 out of 15) and 80% (12 out of 15) horns had at least 1 complete adhesion, respectively (p = .009; 66% reduction) (Fig. 2).

Discussion

Summary of Findings

Our study showed that although the endometrium was thinner on day 1 in the DPF-implanted animals, it did not have a significant impact on endometrial thickness at any of the tested time periods or on pregnancy-related outcomes compared with the control group. In addition, we demonstrated that the DPF was biologically well tolerated, with no evidence that it induced any signs of tissue toxicity. Although the DPF was classified as slightly irritant 24 hours after insertion, this irritation seemed to be short-lived because it did not persist at later assessments. Finally, the DPF significantly reduced the likelihood of complete IUA formation compared with HA gel− and sham-implanted animals, where only 27% of the animals had at least 1 complete adhesion compared with 80% and 100%, respectively. The presence of IUAs in the DPF groups could be due to faster polymer degradation in some rats. Some areas of the horns not covered by the DPF owing to fragmentation may

Table 2

Mean irritation scores of uterine tissues in the DPF and control groups at each interval time (24 hours, 5 days, and 28 days) and calculated irritation index of the DPF

<table>
<thead>
<tr>
<th>Time period</th>
<th>Group</th>
<th>Mean irritation score</th>
<th>DPF irritation index</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>Treated with DPF</td>
<td>3.6 ± 2.95</td>
<td>3.6 (slightly irritant)</td>
</tr>
<tr>
<td></td>
<td>Sham group</td>
<td>0.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>5 d</td>
<td>Treated with DPF</td>
<td>1.6 ± 1.57</td>
<td>1.6 (nonirritant)</td>
</tr>
<tr>
<td></td>
<td>Sham group</td>
<td>0.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>28 d</td>
<td>Treated with DPF</td>
<td>0.0 ± 0.0</td>
<td>0.0 (nonirritant)</td>
</tr>
<tr>
<td></td>
<td>Sham group</td>
<td>0.0 ± 0.0</td>
<td></td>
</tr>
</tbody>
</table>

DPF = degradable polymeric film.
therefore be more susceptible to adhesion formation. In the case of HA gel, and owing to the difference in galenic form (gel vs film), the areas susceptible to adhesion formation would be more numerous, which could explain the higher rate of adhesions in the HA gel group. This observation supports the efficacy of the DPF in preventing IUA formation after intrauterine procedures and concurs with previous reports demonstrating its efficacy in preventing intraperitoneal adhesions [15,16].

Findings in Light of What Is Known

IUAs are an important cause of infertility, implantation failure, miscarriage, and obstetric complications [3]. The exact mechanism of their formation is unknown, and several management modalities have been proposed for their prevention and treatment [14]. Although there are several studies evaluating the impact of IUAs on different clinical outcomes, there is a paucity of information in relation to the efficacy of such interventions on improving fertility [13]. Indeed, although guidelines acknowledge that the use of solid (intrauterine device or balloon) or semisolid (gel) barrier methods reduce postoperative IUA reformation, the use of such barriers is not explicitly recommended, probably owing to the lack of fertility data to justify their recommendation [18]. Furthermore, available barrier agents for the prevention of intraperitoneal adhesions cannot be used in the uterine cavity through the vaginal route [13,19]. It is also important to recognize that adhesions can form anywhere within the uterine cavity; hence, it is imperative that a device intended to reduce such adhesions should cover the entire uterine cavity. Indeed, in this study we were able to confirm the DPF’s efficacy in preventing IUAs at multiple uterine sections within the same horn. Furthermore, our study is quite unique in that it also addressed the local and distant tissue response to the polymer film, which is essential information for the development and licensing of such a medical device. Therefore, we believe that the findings of our study demonstrating the efficacy of the DPF in preventing IUAs, its noninferiority in relation to fertility outcomes, and its minimal biologic effect on the endometrium have a significant clinical potential.

Strengths, Limitations, and Future Implications

We appreciate that our study has some limitations. First, our findings are based on an animal study. Nonetheless, rats are a good model for the evaluation of IUAs and are largely used in similar experimental studies because of the relative ease in managing their reproduction. Furthermore, their bicornuate uterus enables the animal to act as its own control [20]. Therefore, our choice of this model was built on currently available data of similar study designs because demonstrating the DPF’s efficacy in a model with a high tendency for IUA formation will likely be effective in a lower-risk situation such as postmiscarriage curettage [17,20]. Second, in addition to assessing the macroscopic and microscopic effects of the DPF on the endometrium and draining lymph nodes, the evaluation of inflammatory markers would have complemented our results [21].
However, the ability to test the impact of the DPF on endometrial thickness, fertility, biologic responses on surrounding tissues, and IUA prevention in 1 study is a major strength of our work. The natural progression now is to validate our results in humans to assess the efficacy of the DPF on IUA prevention in women. Some adjustments were performed to adapt the polymeric film to the shape and dimensions of the human uterus and to facilitate its transcervical insertion into the uterine cavity while mitigating the risk of any concomitant tissue trauma. This is currently under clinical investigation after hysteroscopic myomectomy to assess the DPF’s efficacy as a preventive barrier against IUAs (NCT04381728).

Conclusion

In conclusion, the DPF has optimal degradation properties, no long-term tissue irritation, and no negative impact on fertility outcomes in rats. Moreover, the DPF significantly reduced the risk of IUA formation in the tested animals compared with HA gel or no treatment. Therefore, the DPF seems to be a potentially promising innovation for the prevention of intrauterine synechiae and their recurrence.

Acknowledgments

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Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.jmig.2020.10.025.

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