Intraamniotic sealing of fetoscopic membrane defects in ex vivo and in vivo sheep models using an integrated semirigid bioadhesive patch

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BACKGROUND: Preterm prelabor rupture of membranes is the most frequent complication of fetoscopic surgery. Strategies to seal the membrane defect created by fetoscopy have been attempted with little success. We previously developed an integrated semirigid bioadhesive patch composed of silicone and hydroxypropyl methylcellulose that achieved ex vivo sealing of membrane defects.

OBJECTIVE: To evaluate the feasibility of the insertion of our integrated semirigid bioadhesive patches using a fetoscopic technique and to test the adhesion in ex vivo human membranes and in an in vivo ovine model.

STUDY DESIGN: An experimental study involving 2 experiments: (1) ex vivo—human fetal membranes were mounted in a custom-designed model with saline solution simulating intraamniotic pressure. The insertion of 2 different bioadhesive patches made of silicone-hydroxypropyl methylcellulose and silicone-polyurethane-hydroxypropyl methylcellulose was performed through a 12-Fr cannula mimicking fetoscopic surgery technique. The experiment was repeated 10 times with membranes from different donors. Measures included insertion time, successful insertion, and adhesion at 5 minutes; (2) in vivo—16 patches of silicone-hydroxypropyl methylcellulose were inserted by fetoscopy in the amniotic cavity of pregnant sheep (4 bioadhesives per animal, in 4 ewes). Measures included successful insertion, adhesion at 5 minutes, and adhesion at the end of surgery.

RESULTS: In the ex vivo insertion study, there was no difference in the insertion time between silicone-hydroxypropyl methylcellulose and silicone-polyurethane-hydroxypropyl methylcellulose patches (P=.49). Insertion was successful in all cases, but complete adhesion at 5 minutes was superior for silicone-hydroxypropyl methylcellulose (P=.02). In the in vivo study, insertion of silicone-hydroxypropyl methylcellulose by fetoscopy was feasible and successful in all cases, and no complications were reported. Adhesion persisted at 5 minutes and at the end of the surgery in 68.8% and 56.3% of the patches, respectively.

CONCLUSION: We describe the feasibility of deploying through a fetoscopic trocar a semirigid silicone-hydroxypropyl methylcellulose patch that seals fetal membranes after an invasive fetal procedure. The results warrant further research for improving long-term adhesion and developing a clinically applicable system.

Key words: adhesion, amniotic fluid, bioadhesive, fetoscopy, hydroxypropyl methylcellulose, iatrogenic preterm prelabor rupture of membranes, insertion, intraamniotic pressure, sheep

Introduction

Fetoscopic surgery is used in thousands of pregnancies worldwide yearly to treat several fetal conditions.[1] One of the main unsolved drawbacks of fetoscopy is the damage incurred to fetal membranes, which results in iatrogenic preterm prelabor rupture of membranes (PPROM) in up to 30% of cases.[1,2] PPROM is the main complication and the main cause of preterm birth and perinatal morbidity after fetoscopy.[1,3]

Several systems have been attempted to seal the fetal membrane defect created by fetoscopy using injectable sealants or patches.[4–6] However, they have shown little success so far. We have previously developed a new concept for fetoscopic membrane sealing by a semirigid bioadhesive patch. The patch is composed of silicone (S) or silicone-polyurethane (SPU), and coated with hydroxypropyl methylcellulose (HPMC) to achieve adhesion to fetal membranes. In a previous study we reported that this system achieved ex vivo sealing of membrane defects, avoiding leakage under high pressure with no cell toxicity.[7]

In this study, we developed and tested the insertion technique for the semirigid membrane patch in human membranes ex vivo and in a fetal sheep model for fetoscopic surgery.

Materials and Methods

Production and characterization of sealing systems

Bioadhesive patches were prepared as previously described.[7] Briefly, patches were produced using either medical S (Nusil, NuSil Technology LLC, Carpinteria, CA) or medical S combined with electrospun SPU (Nusil; Bionate Thermoplastic Polycarbonate Polyurethane, DSM Biomedical, Exton, PA). Both patches were cut in a 17-mm-diameter disk shape, from 450 to 480 μm thick, containing a string used for traction (Aragó 6/0 silk sutures, Laboratorio Aragó, Barcelona, Spain). The surface was prepared with a 150-μm deep micropatterning, created to enhance contact surface and adhesion. Both S

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AJOG MFM at a Glance

Why was this study conducted?

Fetal membrane sealing after fetoscopy remains an unsolved issue. We previously developed a bioadhesive semirigid patch of silicone and hydroxypropyl methylcellulose (S-HPMC) that showed good adhesive properties and low toxicity ex vivo. The feasibility of fetoscopic insertion of this patch should be demonstrated in experimental ex vivo and in vivo models.

Key findings

Insertion of the S-HPMC patch by fetoscopy was feasible and quick. The patch achieved in vivo adhesion in animal models.

What does this add to what is known?

A semi-rigid patch with adhesive properties could be a feasible approach to fetal membrane sealing after fetoscopy. Further research is needed to evaluate long-term adhesiveness and safety.

and SPU were coated with a bioadhesive component of HPMC at 10 mg/mL after plasma activation of S or SPU to form 2 sealing systems: S-HPMC and SPU-HPMC.

The bioadhesive patches containing the traction string were mounted inside a 10-Fr cannula (Check-Flo Performer Introducer, William Cook Europe ApS, Bjæverskov, Denmark). The gray dilator of the kit was adapted to be used as a blunt introducer and the traction string was exteriorized through the dilator to allow adjustments during the insertion (Figure 1). All sealing systems were used up to 30 days after their production. For in vivo studies, sterilization was performed with ethylene oxide.

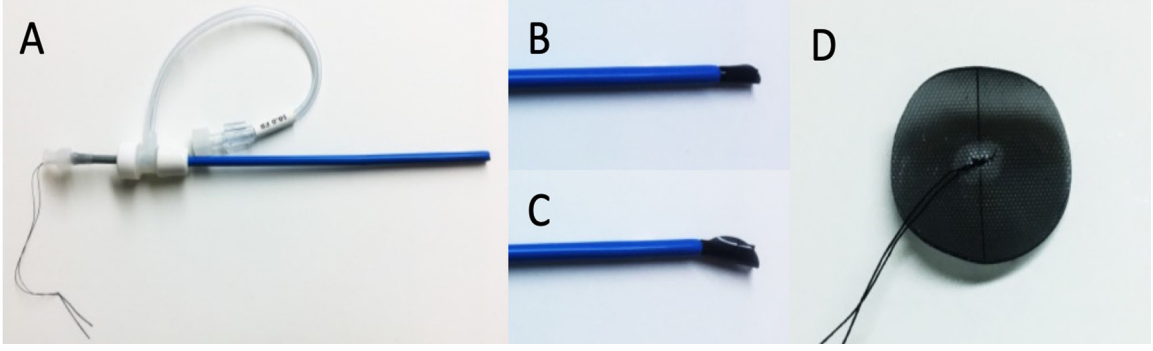
Ex vivo insertion experiments

For the ex vivo experiments, human fetal membranes from 10 donors were collected after cesarean deliveries of singletons at term (from 37−41 weeks' gestation), after written consent. Exclusion criteria were: clinical chorioamnionitis, infections (HIV, hepatitis, syphilis), disturbances of amniotic fluid (oligo or polyhydramnios at the last scan), maternal conditions such as hypertension, diabetes mellitus, anemia, connective tissue disorders, undernutrition, use of tobacco, fetal growth anomalies and major fetal malformations, or chromosomal abnormalities. The study protocol was approved by the ethical committees of the Hospital Clinic Barcelona (HCB/2016/0236) and Hospital Sant Joan de Déu Barcelona (PIC-40-16).

After collection, fetal membranes were separated from the placenta. Areas with visible clots or decidual contamination were removed. Fetal membranes were immersed in saline solution at room temperature and transported to the laboratory within 30 minutes from collection. Explants were cut in 10 × 10 cm pieces at 2 cm from the placental edge to avoid tissue heterogeneity[8] and used within 6 hours. In case the membranes were not used immediately, samples were kept at 4°C until use.

An adaptation of the model used by Mann et al[9] was constructed for the purposes of this study. A 50-mm-diameter, × 150-mm-long transparent cylinder (Phobya Balancer 150 Black Nickel, Aquatuning GmbH, Schloß Holte-Stukenbrock, Germany)—the main cylinder—was modified by cutting, clipping the cut end, and adding a plastic ring to the top. This cylinder was filled with phosphate-buffered saline (PBS) at room temperature. Using a venous line set, the main cylinder was connected to a second plastic graduated cylinder (TPX Graduated Cylinder, Deltalab, Barcelona, Spain) of 41 × 315 mm. The second cylinder was also filled with PBS and placed on a stand and elevated to generate hydrostatic pressure on the first one. A hydrostatic pressure sensor

FIGURE 1
Integrated sealing system



A, Sealing system composed of silicone and hydroxypropyl methylcellulose mounted in a 10-Fr cannula and ready to be inserted. Note the traction string exteriorized through the cannula. **B-C,** Detail: bioadhesive patch being pulled out of the insertion cannula. **D,** Bioadhesive patch with the traction string.

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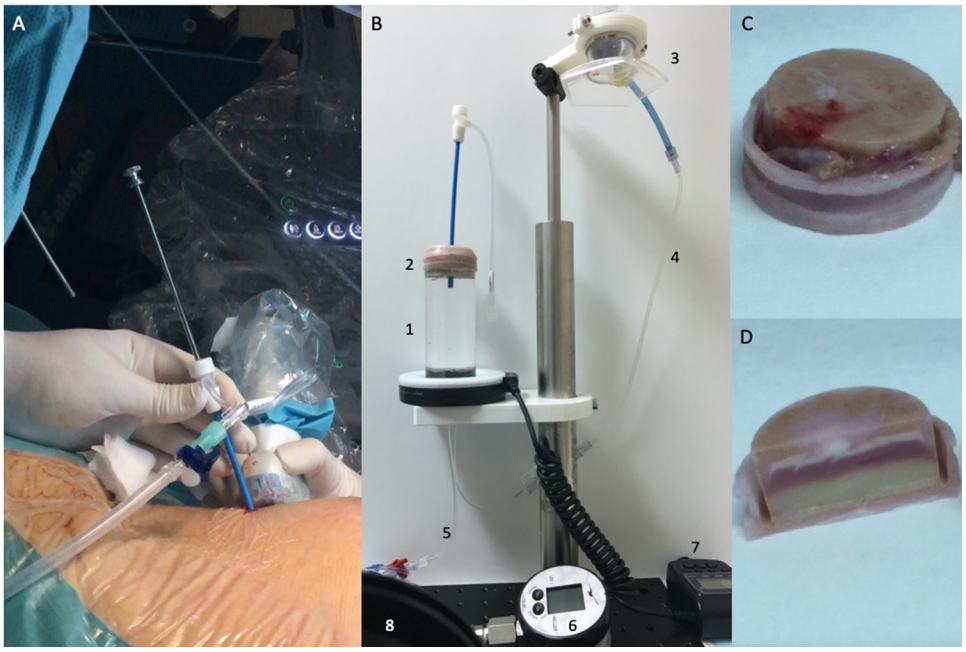
FIGURE 2
Ex-vivo experimental setting



A, Fetoscopic entry with direct technique in the clinical set (cannula and metallic punch). **B,** Experimental set for ex vivo insertion study: (1) main cylinder filled with phosphate-buffered saline and covered with fetal membrane; (2) custom-developed phantom crossed by a trocar (cannula); (3) secondary cylinder (elevated in a stand for generating pressure); (4) inlet tube; (5) outlet tube connected to the pressure senson; (6) pressure sensor; (7) lamp; and (8) lateral camera. **C,** Silicone phantom covered with human fetal membranes. **D,** Lateral sectional view of silicone phantom with the different layers simulating the abdominal wall and uterus of a pregnant woman.

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(digital manometer LEX1 [−1 to 2 bar, accuracy within 0.05%], KELLER AG, Winterthur, Switzerland) was positioned at an outlet tube of the main cylinder. Fetal membranes were mounted on the top of the main cylinder with the amnion facing the inside. To provide a realistic insertion setting, a custom-designed phantom was developed, mimicking the tissue layers of a pregnant woman that are crossed by the trocar during a fetoscopic procedure. It consisted of a 3.7-cm-diameter, 2.2-cm-long silicone piece composed of 5 layers of different textures and densities that simulated the abdominal wall (skin, fat tissue, muscles, fascia) and the uterus. This phantom was applied to fix the fetal membranes on the top of the main cylinder and to offer a resistance to the pressure generated in the system. For this reason, a lateral border of silicone was added to protect the membranes from the cable tie in nylon used for fixation (Figure 2).

The insertion of the sealing system was monitored with a video camera (CMOS Camera [1280 × 1024 px, color, USB 2.0, 18−108 mm EFL zoom lens with focus control, 2/3" format], Thorlabs, Munich, Germany) mounted in front of the cylinder.

After setting the pressure in the main cylinder at 20 mm Hg, a 12-Fr cannula (Check-Flo Introducer Set, RCF-12.0-38-J or G07369, 4.11 mm × 13 cm; Cook Medical, Bloomington, IN) was introduced through the phantom with a direct technique (with a 12-Fr metallic punch inside the cannula). The modified 10-Fr cannula containing either S-HPMC or SPU-HPMC was then introduced through the 12-Fr sheath, and the blunt introducer was used to push the bioadhesive patch into the fluid column. Once the patch was free in the fluid, the sheaths and introducers were removed, and the patch was positioned against the membrane−phantom structure by gently pulling the suture string. Traction was maintained for 2 minutes. After this, the string was withdrawn, and the bioadhesive patch was observed for additional 5 minutes. We measured: (1) insertion time—the time from insertion of the 12-Fr cannula until the attachment of the sealing system to the membrane; (2) successful insertion—if the insertion was possible or not; and (3) adhesion at 5 minutes—started after the removal of the traction string and classified at 5 minutes as complete, partial, or absent. The experiment was repeated 10 times for each group (S-HPMC and SPU-HPMC) with membranes from different donors.

In vivo insertion and adhesion experiments

Animal instrumentation. Four pregnant ewes (*Ovis orientalis aries* breed Ripollesa) were included in the in vivo study. Gestational age was 78 to 90 days (term=145 days) on the day of the first

surgical procedure. The animals were provided by a registered commercial farm (G9900009) and arrived at least 2 weeks before surgery for acclimatization under conventional conditions. The animals had free access to tap water and hay, and standard commercial pelleted diet (Murri rural, Spain) was restricted to 500 g per day per animal. Ewes were fasted the day before the surgery (24 hours for solids, 12 hours for water). Animal handling and all experimental procedures were performed in accordance with applicable regulation and guidelines and with the approval of the Animal Experimentation Ethics Committee of the Universitat de Barcelona (reference, 213.17) and the competent authority Generalitat de Catalunya (reference, 9644).

The animals were subjected to general anesthesia, prepared for sterile surgery, and premedicated (ketamine, 4 mg/kg; xylazine, 0.2 mg/kg; and midazolam, 0.2mg/kg, intramuscular). Induction was performed by intravenous (IV) administration of propofol (4 mg/kg). Preoperative analgesia was performed using a single dosage of buprenorphine (0.02 mg/kg, IV), and cefazoline (1.5 g, IV) was administered as a prophylactic antibiotic therapy. An endotracheal tube (n°8-9) was inserted and mechanical pressure-controlled ventilation was started. Anesthesia was maintained with isoflurane (1.5%−3%) using a semi-closed system. Orogastric and urethral catheters were provided. The animals were placed in left lateral recumbency, and ringer lactate was administered before and during surgery. Heart rate, respiratory rate, oxygen saturation, end-tidal carbon dioxide, and reflexes were monitored and recorded. Body core temperature was maintained using an electric pad. Before starting the surgery, 10 mL of lidocaine (2%) was locally infiltrated at the incision site. Ewes were euthanized using an overdose of pentobarbital (200 mg/kg, IV). Death was confirmed by the cessation of circulation and breathing in both ewes and fetuses. Viability of the fetuses was confirmed by echography before euthanasia. Methodology is reported according to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

Fetoscopy-guided insertion of the sealing system

After general anesthesia, the uterus was exposed through infraumbilical midline laparotomy. Number of fetuses, position, and fetal heart rate (FHR) were verified by ultrasound examination. A 10-Fr cannula was inserted in the ovarian end of the uterine horn by Seldinger technique using a 14-G catheter guided by ultrasound. The cannula was fixed with 2/0 silk stitches, and amnioinfusion of 1.5 mL of warm Ringer's lactate solution was initiated and maintained continuously. A 3.3-mm straight fetoscope 0^0 (KARL STORZ SE & Co KG, Tuttlingen, Germany) was introduced through the 10-Fr cannula and was used to guide and register the insertion of the bioadhesive patches. Two stitches (2/0 silk) were placed laterally to the insertion site to delimit the bioadhesive zone and guide the introduction. The 12-Fr cannula that would receive the 10-Fr cannula containing the bioadhesive patch was inserted by Seldinger technique guided by fetoscopy. The integrated sealing system was applied with the same technique described for the ex vivo phase. After introduction, traction was performed for 2 minutes, and the sealing system was observed for additional 5 minutes after the removal of the traction string. The procedure was repeated to introduce a total of 4 sealing systems in different areas of the selected uterine horn. Before the end of surgery, all sealing systems were checked again for adhesion. We collected information about insertion (possible/not possible) and adhesion at 5 minutes and at the end of surgery (present/absent/partial). In total, 4 bioadhesive S-HPMCs were introduced per ewe, in 4 ewes, resulting in 16 repetitions of the experiment.

Statistical analysis

Categorical variables were expressed as number of cases out of total and proportion (%) and compared using Fisher's exact test. Normal distribution was verified using standardized normal probability plots, box plot graphs, and the Shapiro−Wilk normality test. Parametric numeric variables were expressed as mean±standard deviation (minimum by [maximum range] and compared using Student *t* test. Homogeneity of variances was checked with Levene's test. Data were processed using Stata version 14.2 (StataCorp LP, College Station, TX).

Results

Ex vivo insertion study

Fetal membranes from 10 donors undergoing cesarean delivery were included in the study. Mean gestational age at delivery was 39.4±0.74 weeks. Most common indications were previous cesarean delivery and breech presentation (in 8/10 cases [80%]).

Results of the main outcomes (insertion time, successful insertion, and adhesion at 5 minutes) are shown in Table 1. There was no difference in the insertion time between S-HPMC and SPU-HPMC (*P*=.49). Insertion was successful in all cases, even though complete adhesion at 5 minutes was superior for the S-HPMC bioadhesive

TABLE 1
Insertion and adhesion of bioadhesive patches in the ex vivo model

Variables	N	S-HPMC	SPU-HPMC	*P* value
Insertion time (s)	10	61.3±14.75	67.5±23.27	.49
Successful insertion	10	10 (100)	10 (100)	
Complete adhesion after 5 min[a]	10	10 (100)	5 (50)	.02

Results expressed in mean±standard deviation or number of cases (proportion in percentage). *P* value obtained with *t* test or Fisher exact test.

S-HPMC, silicone-hydroxypropyl methylcellulose; *SPU-HPMC*, silicone and electrospun polyurethane-hydroxypropyl methylcellulose.

[a] (vs partial and absent adhesion).

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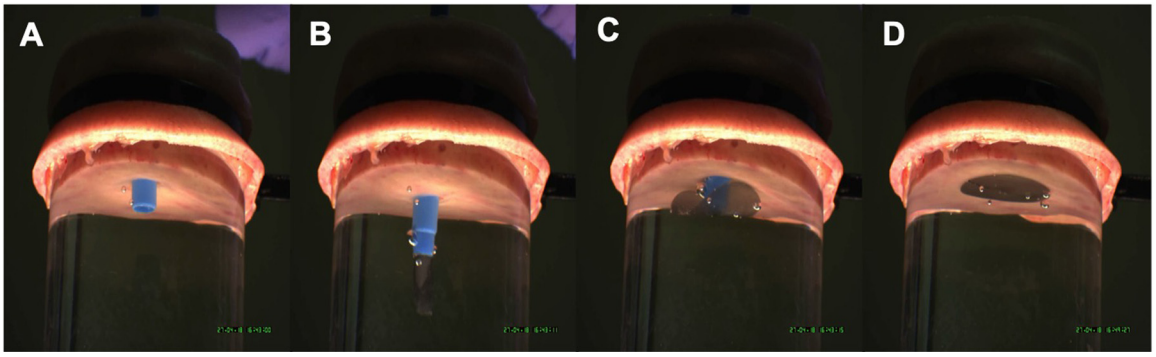
FIGURE 3
Insertion of silicone and hydroxypropyl methylcellulose bioadhesive patch in the ex vivo model



A, Introduction of 12-Fr cannula through the custom-designed phantom and human fetal membrane generating a defect. **B,** Introduction of 10-Fr cannula containing silicone and hydroxypropyl methylcellulose through the 12-Fr cannula. **C,** Sealing system being adjusted to the fetal membrane. **D,** Bioadhesive patch in place sealing the defect generated by the introduction of fetoscopic cannula.

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(*P*=.02). Insertion of the sealing system through the custom-designed phantom and human fetal membrane is represented in Figure 3 and Supplementary Video 1.

In vivo insertion and adhesion study

The mean gestational age of ewes that underwent fetoscopic insertion of the sealing system was 84.3±6.7 days Figure 4. and Supplementary Video 2 illustrate the insertion of the sealing system in the amniotic cavity of a fetal lamb. Insertion of the bioadhesive patches with the fetoscopic technique was feasible and successful in all cases and no complications were reported during the procedure. The sealing system fitted easily to the amnion surface of the fetal membrane. Results of sealing system adhesion at different moments are shown in Table 2. Adhesion was present at 5 minutes and at the end of the insertion surgery in 68.8% and 56.3% of the patches.

Discussion

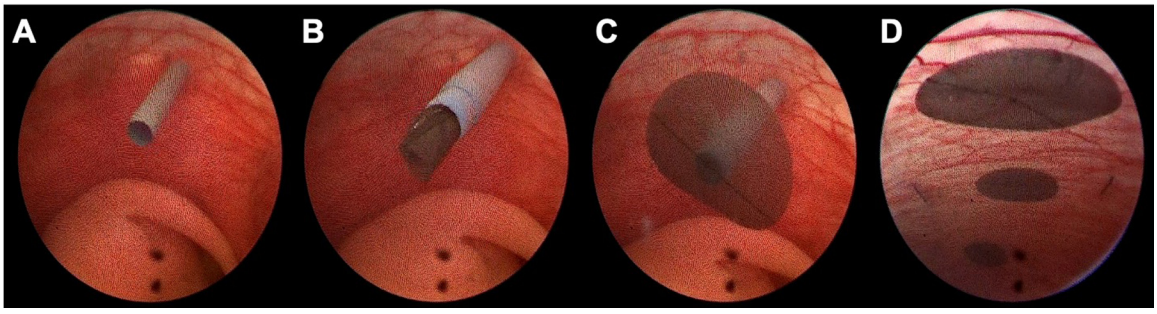
Principal findings

In this study, we report the feasibility of fetoscopic insertion and the short term adhesion to fetal membranes of a previously developed[7] semi-rigid bioadhesive patch for fetal membrane sealing in an experimental setting.

Results

The design of the tested system presents some advantages over previously tested prototypes for membrane sealing. Devaud et al[4] developed a deployable umbrella containing liquid or jelly glue. Despite showing an acceptable adhesion to fetal membranes, the authors reported leakage when injected in wet conditions, whereas the system tested in

FIGURE 4
Insertion of the silicone and hydroxypropyl methylcellulose bioadhesive patch in a sheep model



A, Introduction of 12-Fr cannula through the myometrium and fetal membrane. **B,** Introduction of a 10-Fr cannula containing silicone and hydroxypropyl methylcellulose through the 12-Fr cannula. **C,** Sealing system being adjusted to the amnion surface of fetal membrane. **D,** Three sealing systems in place covering the iatrogenic defect generated by the introduction of fetoscopic cannula.

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TABLE 2
Insertion and adhesion of the bioadhesive patch S-HPMC in pregnant sheep

Variables	Number of bioadhesives inserted (%)
Successful insertion	16/16 (100)
Adhesion at 5 min	11/16 (68.75)
Adhesion at the end of insertion surgery	9/16 (56.25)

Data are shown as number of cases/total number of sealing systems (percentage).

S-HPMC, silicone-hydroxypropyl methylcellulose.

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this study avoids liquid components. We tested insertion through a fetoscopic trocar, which eliminates the need for external instruments like tweezers or sutures. Other studies have reported the experimental use in fetal membranes of an adhesive patch developed for other types of open or endoscopic surgeries (ie, thoracic surgery).[5] However, such a system could not be applied for fetal membranes through a single-port trocar, which may restrict its use for fetal surgery. The system tested in this study was conceived specifically to represent a ready-to-use system that can be inserted through a fetoscopic port. Finally, other methods previously described to seal fetal membranes have been based on collagen plugs.[10] However, such plugs are by definition positioned through the membrane layers, which probably disturbs the sliding of the amnion over the chorion[11] and may explain why they have showed no benefit when used to prevent PPROM.[10]

Clinical and research implications

The concept of introducing a semirigid, nonabsorbable integrated patch on the amnion surface of fetal membranes after fetoscopic surgery is original and easily translated to clinical use. The insertion time in this study was 1 minute, and therefore the technique adds minimal additional time to the fetoscopic procedure. The results showed promising short-term adhesion that remains to be improved through further developments of the patch characteristics. Achieving tissue adhesion to a slippery biological tissue such as the amnion in a wet environment remains the main challenge. If successful, the application would improve perinatal results of current fetoscopic surgery.

Strengths and limitations

Among the strengths of this study is its experimental nature, which allowed us to test different aspects of the bioadhesive patches in controlled settings. First, the ex vivo model takes in consideration the intrauterine pressure, and the custom-designed phantom offers an appropriate resistance to the insertion, imitating the uterine wall and other maternal tissues. Second, the sheep model adds the advantage of using fetoscopic visualization of the insertion and evaluating adhesion in "live" tissue. In contrast, the experimental nature of this study also represents a limitation. Although the ovine model contributes to evaluating insertion and adhesion of the bioadhesives patches, it is not a reliable model of PPROM, given that the rupture of membranes with amniorrhexis seems to be a condition occurring mainly in humans and other primates.[12] In addition, anatomic differences between sheep and humans, such as the thickness of the myometrial wall and the vascularized chorioamnion, may limit the comparability of the experimental results with the clinical situation in human pregnancies. Another limitation is that the sheep study design did not allow histologic evaluation of toxicity or long-term assessment of adhesion. Furthermore, we used term membranes in the ex vivo study and, although there are no remarkable histologic differences between term and preterm membranes,[13] it could be argued that adhesiveness could be lower in the latter, especially because of differences in stiffness.[14]

Conclusion

Our results showed the feasibility of fetoscopic insertion of an integrated bioadhesive patch that seals the membrane defect after fetal surgery. Further studies are needed for improving long-term adhesion to advance toward a clinically applicable system. ■

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ajogmf.2022.100593.

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