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Cryopreserved human amniotic membrane and a bioinspired underwater adhesive to seal and promote healing of iatrogenic fetal membrane defect sites

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ABSTRACT

Introduction: We investigated the ability of cryopreserved human amniotic membrane (hAM) scaffold sealed with an underwater adhesive, bio-inspired by marine sandcastle worms to promote healing of iatrogenic fetal membrane defects in a pregnant swine model.
Methods: Twelve Yucatan miniature pigs underwent laparotomy under general anesthesia at 70 days gestation (term = 114 days). The gestational sacs were assigned to uninstrumented (n = 24) and instrumented with 12 Fr trocar, which was further randomized into four different arms-no hAM patch, (n = 22), hAM patch secured with suture (n = 16), hAM patch with no suture (n = 14), and hAM patch secured with adhesive (n = 9). The animals were euthanized 20 days after the procedure. Gross and histological examination of the entry site was performed for fetal membrane healing.
Results: There were no differences in fetal survival, amniotic fluid levels, or dye-leakage from the amniotic cavity between the groups. The fetal membranes spontaneously healed in instrumented sacs without hAM patches. In sacs with hAM patches secured with sutures, the patch was incorporated into the swine fetal membranes. In sacs with hAM patches without sutures, 100% of the patches were displaced from the defect site, whereas in sacs with hAM patches secured with adhesive 55% of the patches remained in place and showed complete healing ($p = 0.04$).
Discussion: In contrast to humans, swine fetal membranes heal spontaneously after an iatrogenic injury and thus not an adequate model. hAM patches became incorporated into the defect site by cellular ingrowth from the fetal membranes. The bioinspired adhesive adhered the hAM patches within the defect site.

1. Background

Despite the recent advances in invasive fetal surgeries have improved fetal outcomes, iatrogenic preterm premature rupture of membranes (iPPROM) remains a major complication leading to premature delivery undermining the complete benefits of such surgeries [1,2]. iPPROM has been attributed to the non-healing nature of human fetal membranes [3,4] and chorioamnion separation at the site of entry [5]. Numerous *in vitro* and *in vivo* efforts to repair iatrogenic fetal membrane defects have been reported using various sealants and plug materials, including fibrin based products [6], collagen slurries, blood cryoprecipitates, platelets [7], collagen sponges, and decellularized tissue scaffolds [8,9]. *In vitro* experiments suffered from poor experimental design with respect to replicating the wet amniotic environment. Previous *in vivo* studies

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conducted in rabbits were limited in their ability to study complete wound healing due to the short 28 day gestation period. In our retrospective studies, plugging the fetoscopic entry port with gelatin sponge material after laser surgery did not decrease the incidence of iPPROM compared to laser surgeries in which sealants were not used [10,11]. There is no proven method available for humans that reduces the incidence of iPPROM.

Cryopreserved human amniotic membrane scaffolds (hAM) have been used in ophthalmology as a permanent graft to fill in tissue defects that allowed integration of host cells into the defect, and as a temporary biological bandage to facilitate wound healing by suppressing excessive surgical or disease-induced host tissue inflammation [12]. Due to hAM's inherent anti-inflammatory and anti-scarring properties, it has been used in orthopedic applications to decrease local inflammation and adhesion formation following tendon [13,14] and nerve repair [15].

The water-borne adhesive used in this study was inspired by the undersea glue of sandcastle worms [16]. To create a synthetic biomimetic adhesive, the chemistry of the natural glue was mimicked with sets of oppositely charged polyelectrolytes synthesized with the same side chain chemistry (phosphates and primary amines) in the same molar ratios as the natural glue proteins [17]. The bioinspired adhesive has several ideal properties as an injectable wet-field adhesive. Most importantly, the oppositely charged PEs associate electrostatically and condense into a concentrated fluid macrophase in a narrow range of solution conditions. Although the individual polyelectrolytes components are highly water soluble, the condensed polyelectrolyte macrophase is slowly miscible with water, and therefore does not dissolve or disperse into physiological fluids, including blood [18] and amniotic fluid, on a time scale of hours. As a result, the water-borne adhesive remains at the application site during the curing process even when fully submerged in water. In a previously published study, the feasibility of using the synthetic adhesive with hAM scaffolds to seal wet and submerged fetal membrane defects was demonstrated i*n vitro* [19]. In that study, the synthetic adhesive was shown to be non-cytotoxic using live *ex vivo* human amniotic membranes. A similar condensed polyelectrolyte adhesive formulation was biocompatible and effectively secured and maintained alignment of rat skull fragments during healing [20].

In this study, we used a swine model, which has gestational age of 114 days, to observe changes at the trocar site between 18 and 21 days post-surgery to understand the process of wound healing. Two hypotheses were tested in two different phases of study, Phase I: hAM patches promote fetal membrane healing after an iatrogenic defect compared to no hAM patch, and Phase II: underwater adhesive stabilized the hAM patch at the site of defect to promote healing.

2. Materials and methods

2.1. Human amniotic membrane scaffold and underwater adhesive

Research grade hAM was kindly provided by Bio-Tissue, Inc. (Miami, FL) [19]. The adhesive was prepared as previously described [21]. The details of the methods of preparation are in the supplementary material.

2.2. Animal study

The study protocol was approved by the Institutional Animal Care and Use Committee (AWC-12-038) at The University of Texas Health Science Center at Houston. All animal care was in compliance with the Guide for the Care and Use of Laboratory Animals. The animal facility is accredited by Association for Assessment and Accreditation of Laboratory Animal Care – International, and the Public Health Service Policy on Humane Care and Use of Laboratory Animals. The facility meets all standards mandated by the Animal Welfare Act, Centers for Disease Control, National Research Council Guide for the Care and Use of Laboratory Animals.

Pregnant Yucatan miniature swine were obtained from Sinclaire Bio-Resources (Columbia, MO). Time-mated, pregnant Yucatan swine arrived at the facility one week prior to surgery for acclimatization. The animals were cared for by trained and experienced veterinary technicians supervised by board-certified veterinarians. The animals had free access to food and water except for the 12-h period directly preceding surgery. The surgery was performed at 70 days gestation (term: 114 days) under general anesthesia using isoflurane inhalation. The list of medications for pre-, intra- and post-operative periods are listed in Table 1. Indomethacin was administered as a rectal suppository before surgery and intra-operative terbutaline was given by intravenous pump for tocolysis. Medroxyprogesterone acetate intramuscular injections were given as a supplement for 4 days to prevent post-surgical luteal deficiency.

At the time of the surgery, the pregnant sow was placed in the left lateral position to prevent compression of the inferior vena cava. Local anesthesia was administered with a subcutaneous injection of 20 cc of 0.25% bupivacaine on the right side lateral to the mammary glands followed by vertical laparotomy incision. The uterine horns were exposed with gentle manipulation. The gestational sacs that were closest to the cervix were not instrumented (negative controls). The remaining sacs were instrumented. The surgeries were performed in 2 study phases. In phase I, the gestational sacs from 6 animals were randomized into two groups: instrumented without a hAM scaffold placement, and instrumented with a hAM scaffold secured in the defect with sutures. In phase II, the sacs were randomized into two groups: instrumented with hAM scaffold placement without sutures, and instrumented with hAM scaffold placement secured in the defect with adhesive.

The instrumentation to enter the amniotic cavity for all sacs was performed in the similar manner as in humans for percutaneous approach during a fetoscopy [10,22]. Under ultrasound guidance the entry site was chosen on the *antimesenteric* side of the uterine horn to avoid the allantois. An 18 gauge echo tip needle (Cook; Bloomington, Indiana, USA) was inserted into the gestational sac under ultrasound guidance. This was followed by J-wire insertion, after which a 12 Fr trocar (Cook; Bloomington, Indiana, USA) and cannula were threaded over the J-wire. The entry into the gestational sac was confirmed by the back flow of amniotic fluid through the cannula. Ten cc of amniotic fluid from each instrumented sac was collected.

For the instrumented with hAM placement sacs, a 4 by 4 cm square patch of hAM scaffold was folded into an “umbrella” shape with the chorionic surface facing out [19]. The optimal patch size to occlude the 12 Fr cannula and the method of delivery has been described in our previous publication [19]. A 4-0 monocryl suture was tied to the center of the fold. The hAM patch was loaded into the 10 Fr cannula with a 8 Fr blunt trocar to push the patch. The unit was introduced into the 12 Fr operating cannula and the patch was displaced into the amniotic sac. Both cannulas and the trocar were withdrawn leaving behind the hAM patch in the amniotic sac with the retrieval suture coming through the uterine entry site. Gentle traction was applied to the suture to draw the hAM patch into the trocar defect site.

In instrumented sacs sealed with hAM and sutures, a 4-0 monocryl suture was placed through the uterine wall into the patch to secure it to the uterine wall. In instrumented sacs sealed with hAM without sutures, the excess retrieval suture was cut close to the patch and no fixation was used. In instrumented sacs sealed with hAM and adhesive, part of the patch was pulled into the incision and the activated adhesive was applied between the patch and the uterine wall. The retrieval suture was cut close to the patch.

Table 1
List of medications for the surgery.

One day before surgery:
- Medroxyprogesterone 50 mg IM
- Indomethacin 50 mg rectal suppository

Day of surgery: **All drugs given are calculated on non-pregnant weight (minus 10 kg)**

Pre-operatively
- EMLA cream (lidocaine 2.5% and prilocaine 2.5%) applied to injection sites 30–45 min prior to sedation to minimize discomfort
- Telazol (tiletamine/zolazepam 100 mg/ml) 4–8 mg/kg IM
- Famotidine 0.25 mg/kg SC
- Naxcel (ceftiofur sodium) 3 mg/kg IV
- Lactated Ringers 10 ml/kg/h IV
- Duramorph (morphine) 1 mg in 10 ml of normal saline epidural

Intra-operatively
- Terbutaline 2.5mcg/min IV infusion
- Bupivicaine 0.25% 20 ml for subcutaneous injection
- Isoflurane 2–3% inhalation

Post-operatively
- Naxcel (ceftiofur sodium) 3 mg/kg IV
- Excede (Ceftiofur Crystalline Free Acid 100 mg/ml) 5 mg/kg IM
- Buprenorphine 0.01 mg/kg subcutaneous injection

Post-op Day 1 – 4:

- Buprenorphine 0.01 mg/kg subcutaneous injection q8 hours × 1 day, PRN thereafter
- Medroxyprogesterone 50 mg IM on Day 2 and Day 4

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