

Bioengineering of foetal membrane repair

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Summary

Preterm premature rupture of the foetal membranes (*früher vorzeitiger Blasensprung*) has remained a devastating complication of pregnancy with very high risk of pregnancy loss. Several methods of sealing spontaneously ruptured membranes to stop amniotic fluid leakage and prolong pregnancy have been tested, but no one of them has achieved a clinical breakthrough. Also, needle and foetoscopic puncture of membranes for diagnostic or surgical interventions in the amniotic cavity carry a significant risk of persistent membrane leakage and subsequent rupture – thus limiting the developing field of intrauterine foetal surgery. Efforts are concentrated on taking action *before* rupture rather than reacting *after* rupture: one avenue of research concerns prophylactic plugging of foetoscopic access sites in foetal membranes at the time of intervention, thus in-

hibiting leakage and rupture. Foetal membrane injuries, spontaneous or iatrogenic, constitute extreme challenges to repair: thinness of foetal membrane tissue, difficult localisation and accessibility of the rupture site, the need for injectable sealants, wet gluing conditions and poor wound healing in this tissue all complicate repair. The goal is to achieve immediate and at the same time long-lasting closure of the membrane leak. Here we review approaches to closure of foetal membrane defects with liquid sealants or solid biomaterial scaffolds, with the focus on prophylactic plugging of foetoscopic access sites.

Key words: spontaneous PPROM; iatrogenic PPROM; tissue sealants; foetoscopy, injectable hydrogel; scaffolds

Introduction

The foetal membranes, consisting of amnion and chorion, constitute an ~0.5 mm thin layer surrounding the amniotic cavity. They hold the amniotic fluid and guard the foetus against infection ascending the genital tract [1]. Mechanical rupture of the membranes is part of the natural sequence of term delivery, but has serious complications when rupture occurs prior to term; preterm premature rupture of the foetal membranes accounts for some 30% of premature births [2]. Histology indicates that the membranes possess a morphologically predefined “breaking site” over the cervical ostium for rupture [3, 4]. However, this site appears prone to spontaneous rupture before the onset of labour. Spontaneous preterm premature rupture of the membranes (sPPROM) before 37 weeks of gestation in the absence of labour affects 1–4% of pregnancies and accounts for 30–40% of preterm deliveries [5, 6]. It usually occurs in the second half of pregnancy. Rescue of pregnancy demands immediate sealing of the amniotic sac. Though several methods of sealing exist (see below), none

have been found to be really effective. Current management of sPPROM is immediate delivery or termination of pregnancy. In surviving infants morbidity is often severe and includes chronic lung disease and neurological sequelae such as blindness, cerebral palsy, white matter damage and periventricular leukomalacia. Intrauterine infection and inflammation are the likely triggers of sPPROM, but the pathological sequence leading to rupture of the membranes is not well understood. At the molecular level, sPPROM appears to be the result of diminished extracellular matrix (ECM) synthesis, alterations in the structure of collagen fibrils and increased ECM degradation. A point to note is that it is not clear whether infection and inflammation precede defects in the extracellular matrix or vice versa.

Diagnostic or surgical invasions of the amniotic cavity with needles or foetoscopes pose a risk of persistent leakage and subsequent premature preterm rupture of foetal membranes (iatrogenic PPROM; iPPROM). The most frequent invasion in clinics, mid-trimester genetic amniocentesis, is

The authors acknowledge support by the Swiss National Science Foundation grant no. 31000-108270; EU Commission FP6-grant “EuroSTEC” (European programme for soft tissue engineering for congenital birth defects in children (<http://eurostec.tv.nl>); and the Zurich Centre for Integrative Human Physiology.

associated with rupture in only a small number (approx. 0.5%) of patients. Hence punctures from amniocentesis are certainly no targets for prophylactic plugging, but plugging could be tried in those cases where leakage after amniocentesis persists. In some cases rupture after amniocentesis seems due not to the intervention itself but to a preexisting subclinical intraamniotic infection. Several studies have shown that a foetal inflammatory response, defined by elevated amniotic fluid levels of pro-inflammatory cytokines (e.g. interleukin-6), matrix metalloproteinase-8 or C-reactive protein at the time of amniocentesis are associated with an increased risk of spontaneous preterm delivery [7–10].

Thanks to advances in fiber endoscopes, operative foetoscopy on foetus and placenta has become a therapeutic option [6, 11]. Examples are foetoscopy-guided laser coagulation for foeto-foetal transfusion syndrome, umbilical cord ligation, and percutaneous endoluminal tracheal occlusions for isolated congenital diaphragmatic hernia. These invasive procedures are usually performed in the second trimester of pregnancy. A

common complication is persistent leakage of amniotic fluid from punctured sites, which may result in preterm delivery [12]. Despite the fact that these procedures are usually performed via a single intrauterine entry, rupture from puncture occurs in 5–30%. Hence iPPROM presents a serious limitation for the developing field of intrauterine foetal surgery.

The technical as well as biological obstacles for closure of injured foetal membranes are manifold and extreme: thinness of the foetal membrane tissue, difficult localisation and accessibility of the rupture site in the uterine cavity, large lesions in the several-centimeter range, poor demarcation of wound edges, the need for injectable sealants, wet gluing conditions etc, complicate repair. Moreover, histology indicates that wound repair is poor or absent in foetal membrane tissue [13], which adds to the difficulty of obtaining stable integration of material plugs grafted into ruptured membranes over weeks or months. Here we review experimental and early clinical attempts to close and prevent ruptured foetal membranes.

Characteristics of foetal membrane wounds

Iatrogenic and spontaneous foetal membrane wounds differ in location and appearance (see [5] for comprehensive review) and therefore also in

the technical routes for their access and sealing. Illustrations of Figures 1A–C reflect current evidence. Endoscopic visualisations of spontaneous

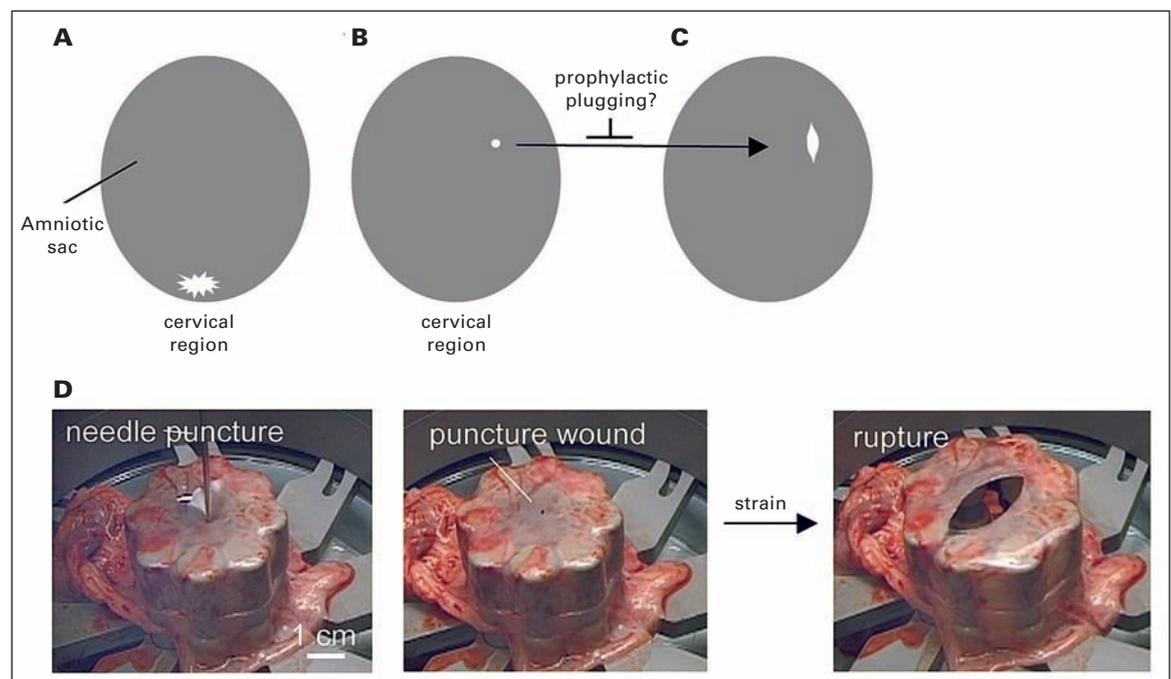


Figure 1

Characteristics of foetal membrane wounds on current evidence. (A) Spontaneous membrane ruptures seem to occur at a predefined, natural breaking site in the cervical region of the foetal membranes [4]. Spontaneous ruptures are poorly demarcated wounds in the several-centimeter range. (B, C) Iatrogenic wounds. (B) Foetoscopy access leads to small-sized wounds in the few-millimeter range in foetal membranes. The wound site is only known at the time of the intervention. (C) Rupture from iatrogenic puncture. According to [5], iatrogenic ruptures may be more cleanly delineated than spontaneous ruptures. Prophylactic plugging of foetoscopy access sites may inhibit membrane leakage iatrogenic rupture. (D) Ex vivo simulation of iatrogenic rupture from puncture. A needle puncture wound of 1.4 mm diameter was created in fresh foetal membranes collected from a term pregnancy. Upon mechanical stretching, the foetal membranes rupture from the site of the puncture. A cleanly delineated wound of several centimeters in size develops.

rupture sites in humans show large, torn wounds in the several-centimeter range over the cervical ostium [14], possibly corresponding to the predefined 'breaking site' in the cervical region of foetal membranes [4] (fig. 1A). Wound edges were found to roll, resulting in irregular wounds that will be even more difficult to seal; this adds another reason for avoiding any time lapse between rupture and sealing. Iatrogenic ruptures in the foetal membranes locate to needle insertion sites. According to [5], ruptures from punctures are smaller and more cleanly delineated than spontaneous ruptures (fig. 1C). Histological followup indicates that puncture wounds in the foetal membrane tissues do not close by growth of new tissue [13, 15]. Rather, spontaneous closure can be attributed to sliding of amnion and chorion relative to each other, contraction, or reattachment of the foetal membranes to the decidual layer of the

uterine wall [13]. The size of iatrogenic membrane defects, and thus the chance of rupture, increase with instrument size. The initial defect size may range from a typical 20–22 gauge perforation in the case of amniocentesis up to a 2–5 mm stab wound from trocars used in operative foetoscopy [16]. Foetoscopic punctures therefore carry a significantly higher risk of rupture [5]. Figure 1D shows an *ex vivo* simulation of iatrogenic rupture in fresh human foetal membranes from a 1.4 mm puncture after mechanical stretch. In this dramatic case, ripping of the foetal membranes from the puncture site resulted in a cleanly delineated wide open wound of several centimeters in length, which at this point has probably become impossible to reseal. Plugging of foetoscopic access sites at the time of intervention, as illustrated in fig. 1B, may inhibit leakage and subsequent rupture.

Methods of closure *after* rupture

Since the early 1990s, clinical attempts have been made to stop fluid leakage after sPPROM or iPPROM by physically plugging the cervix or the membrane defect with liquid sealants or solid scaffolds. An appealing and clinically practical strategy was to inject blood clotting agents to form a blood clot patch in the cervix or directly at the lesioned membrane *in situ* [17]. For example, sealing of sPPROM was approached by delivery of maternal platelets mixed with fibrinogen solution via intraamniotic instillation or foetoscopic application ("amniopatch" [18]), and intracervical application of a clinical off-the-shelf preparation of "fibrin glue" [19]. One centre in the US reported an approximately 50% success rate with "amniopatch" sealant in the treatment of iPPROM [20], but these data await confirmation from other centres. Young et al. reported successful closure of post-amniocentesis ruptures by endoscopy-guided sequential injection of platelets, fibrin, and powdered collagen slurry in 3 of a total of 4 human cases [12]. Scaffolds have also occasionally been tried for closure of spontaneously

ruptured membranes in human cases with highest risk of pregnancy loss. This includes one case of endoscopic placement of a collagen plug ("amniograft") over the membrane defect over the internal os for sPPROM at gestational week 17 [21], or cervical plugging with a gelatin sponge for sPPROM before 21 weeks [22]. In the case of amniograft, leakage recurred after 14 days but the pregnancy continued till week 30. In the gelatin sponge study 8 of 14 fetuses survived but showed musculoskeletal abnormalities [22]. Although early clinical experience indicates the feasibility of treating iPPROM, no treatment has been implemented in clinics. New material innovations for sealing remain in great demand in the field. Key requirements for closure are low resorption and long-term engraftment of tissue adhesives and scaffolds respectively. There are no animal models for spontaneous rupture of foetal membranes, in contrast to iatrogenic rupture (see below). New treatment solutions validated for iPPROM may be tried subsequently for spontaneous PPROM.

Methods of closure *before* rupture

The overall objective is to minimise the risk of membrane rupture from puncture. One avenue of research concerns prophylactic sealing of foetoscopic access sites in foetal membranes in order to inhibit amniotic fluid leakage and iPPROM. The idea is to deploy the plug material into the puncture site before the fetoscope is finally retrieved. The method of sequential injection of platelets, fibrin glue and powdered collagen slurry used after membrane rupture [12] has also proved

successful for prophylactic treatment. In a small clinical study by Young et al., injection of such a mix directly to the puncture site successfully prevented amniotic fluid loss after an endoscopic procedure [23]. To date, however, most experience is preclinical and has been largely acquired in the rabbit model at midgestation. Though the relationship of the foetal membranes to decidua and myometrium in rabbits differs greatly from that in humans, surgically induced membrane defects

Table 1
Plugs for closure of
foetoscopic access
sites.

Study	Plugging material	Animal	Improved amniotic integrity
Papadopulos et al. (1998) [16]	Collagen (Colgen®)	rabbit	No
Deprest et al. (1999) [24]	Collagen (Colgen®)	rabbit	No
Luks et al. (1999) [26]	Gelatin sponge (Gelfoam) + myometrial suture	sheep	All foetuses survived till the ewes were killed near term
Luks et al. (1999) [26]	Gelatin sponge (Gelfoam) + myometrial suture	rhesus monkey	3 out of 5 foetuses survived till spontaneous delivery
Gratacos et al. (2000) [25]	Collagen (Colgen®) + myometrial suture	rabbit	Yes
Gratacos et al. (2000) [25]	Matrigel + myometrial suture	rabbit	No
Devlieger et al. (2003) [36]	Porcine small intestine (bioSIS) contains transforming growth factor- β and basic fibroblast factor	rabbit	Yes
Papadopulos et al. (2006) [27]	Collagen (TissueFoil E®) + fibrin sealant + myometrial suture	rabbit	Yes
Papadopulos et al. (2006) [27]	Tissue banked human amnion membrane + fibrin sealant + myometrial suture	rabbit	No
Papadopulos et al. (2006), Mallik et al. (2007) [15, 27]	Collagen foil (TissueFleece®) + fibrin sealant + myometrial suture	rabbit	Yes
Mallik et al. (2007) [15]	Decellularized human amnion + fibrin sealant + myometrial suture	rabbit	Yes
Ochsenbein-Kölbl et al. (2007) [32]	Decellularized rabbit amnion + myometrial suture	rabbit	Yes
Ochsenbein-Kölbl et al. (2007) [32]	Polyesterurethane (Degrapol)	rabbit	Yes

that are left uncovered will persist in most cases and lead to oligohydramnios and its implications for foetal development. Amniotic integrity, amniotic fluid volume and foetal survival are the main parameters for measuring treatment performance. Solid scaffolds made of collagen, the main constituent in the ECM of foetal membranes, and also synthetic polymer plugs were tested in this model (table 1). In 1999, Deprest et al. demonstrated the technical feasibility of foetoscopically deploying collagen plugs for coverage of foetoscopic puncture defects on surgically exposed amnion sacs in the rabbit model [24]. Thereafter, foetoscopy-guided insertion of solid collagen plugs into the punctured membrane ('champagne

cork method', see fig. 2), sealing with fibrin, and surgical closure of myometrium by suture were tried as individual methods and in combination to restore amniotic integrity after foetoscopy. Each method alone did not exhibit any significant benefit for restoration, but the combination of collagen plug with myometrial suture finally proved successful [25]. Importantly, the latter technique applied with a gelatin sponge also proved effective for long-term closure of foetoscopic access sites in sheep and rhesus monkeys, which have gestational periods of 145 and 165 days respectively [26]. Endoscopy showed that the inserted gelatin sponge rapidly swells through fluid absorption and self-locks in the membrane defect.

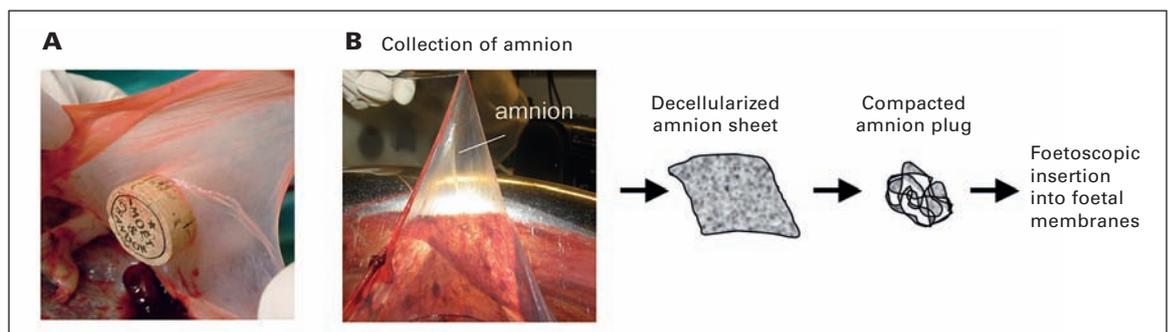


Figure 2

Plugging foetoscopic access sites in foetal membranes with scaffolds. (A) The prophylactic plugging approach, here illustrated with a champagne cork inserted into a large foetal membrane defect, aims to create a physical barrier to amniotic fluid. (B) Deployment of plugs made of decellularized human amnion is a potential practical surgical method to seal foetoscopic access sites [15, 32]. Human amnion is a readily accessible, unique source of human collagen matrix. The 0.2 mm thin, blood vessel-free amnion is an ideal tissue for decellularisation which renders human amnion non-immunogenic. The decellularised amnion sheet is inserted as a compacted plug that self-locks in the foetal membrane wound.

The common notion of low or absent anatomical repair and poor plug engraftment in foetal membranes has prompted biological research to activate repair. We, like others, have considered supplying growth factors or amnion cells in the graft material to enhance cell migration and graft colonisation, i.e. a concept of tissue engineering for foetal membrane repair [27–30]. In line with this idea, we used collagen gel and fibrin gel matrices seeded with amnion epithelial and mesenchymal cells to mimic natural amnion tissue *in vitro* [29]. This showed fibrin gel to be principally suitable for amnion cell grafting, but it is resorbed too quickly. Collagen gels seeded with amnion mesenchymal cells showed dramatic contraction, which would probably cause rapid loss of the plug from the defect. As an alternative we introduced decellularised collagen scaffolds produced directly from human amnion, with the idea that native matrix might integrate better [15, 31, 32]. Human amnion tissue constitutes a readily accessible and unique source of human ECM. Because the amnion layer of human foetal mem-

branes is only 0.2 mm thin and lacks blood vessels, it is an ideal starting tissue for manufacture of sheets of cell-free ECM. It is well established that removal of cellular components renders allogeneic and xenogeneic tissue non-immunogenic [33]. Figure 2 shows the sequence of amnion plug preparation. Two independent trials in rabbits suggest that foetoscopic deployment of decellularised amnion plugs to be a surgically practical method for closure of foetoscopic access sites [15, 32]. Once released from the fowetoscope into the lesion, the compacted plug relaxes and self-locks into the lesion. There was no evidence of incipient anatomical remodeling of the plug into foetal membrane tissue. Decellularised amnion plugs involve several benefits for applications in the clinic, as they exhibit superior surgical handling characteristics, are easy to manufacture, are non-immunogenic and biocompatible, and are suitable for storage and transport for off-the-shelf use. Further studies are necessary to validate the safety and long-term functionality of cell-free amnion plugs.

Limitations

Major limitations concern the animal models for foetal membrane repair. The anatomy of foetal membranes in human and non-human primates is unique. Potential maternal problems arising from intervention with plugs, such as adhesions, are likely to be absent in the rabbit and sheep models. In the case of prophylactic plugging, the human situation requires the plug to be applied by percutaneous needle foetoscopy, and to last in the defect for several weeks or months. But neither percutaneous needle foetoscopy nor long-term fol-

lowup is possible in the rabbit model (as discussed by us in [15]). Hence studies in non-human primates or sheep with longer gestational periods will be needed to resolve whether durable sealing with plugs can be achieved. Stable integration and a low resorption rate are all areas of research for the near future. Foetoscopic grafting of solid plugs may be suitable for closure of focal defects in the foetal membranes, but probably not for larger, undefined defects as produced on spontaneous PPRM.

Conclusions

For biological and technical reasons foetal membrane wounds are extremely difficult to treat. So despite the magnitude of this problem in obstetrics, only a few centres worldwide have taken up the challenge of foetal membrane repair. However, prevention of rupture from puncture by plugging at the time of foetoscopic intervention appears to be feasible. In addition to solid plugs of collagen or cell-free amnion matrix, innovations in the field of injectable, *in situ*-forming synthetic polymer hydrogel sealants offer new options for sealing of iatrogenic puncture wounds. These include the bioinspired DOPA-based hydrogel mimetics of mussel adhesives [34] or photopolymerisable poly(ethylene glycol) hydrogel barriers [35]. It remains to be seen whether such polymeric hydrogel sealants can fulfill their barrier function long-term on fast growing human foetal

membranes. The emerging evidence seems to militate against the concept of regenerating defective foetal membranes at the anatomical level. However, studies in non-human primates or sheep are needed to resolve whether the concepts of mere physical plugging are more advantageous than the “living tissue engineering concept”. We believe that the future selection criteria for candidate sealants for foetal membrane repair may be narrowed to their potential as an immediate, stable, non-toxic physical barrier to amniotic fluid.

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