

Amnion-on-a-Chip: A Microfluidic Platform for Modeling Fetal Membrane Barrier Function and Drug Transport

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Abstract

Fetal membrane rupture during in utero procedures can lead to amniotic fluid leakage and adverse pregnancy outcomes, yet current in vitro models cannot simultaneously evaluate intact and damaged membrane transport. In this study, we developed a microfluidic Amnion-on-a-chip platform consisting of orthogonal PDMS channels separated by a nanoporous polycarbonate (PCTE) membrane to model transmembrane transport under controlled flow conditions.

Device integrity was validated through qualitative dye testing and quantitative FITC measurements, confirming a robust, leak-free seal with transport restricted to membrane diffusion. Baseline experiments demonstrated diffusion-dominated transport in the intact membrane, with cumulative FITC increasing over time and transport rates scaling with flow at lower conditions. Deviation at higher flow rates suggested the onset of convective effects, defining an upper limit for purely diffusion-driven transport. Variability in back-calculated effluent concentrations suggests that total transported FITC and transport rate are more reliable metrics than concentration in this system.

Although post-puncture transport could not be quantified due to a lack of induced flow, this platform establishes a reliable baseline for intact membrane behavior and provides a foundation for future optimization to model puncture-induced leakage. This system has potential applications in studying membrane repair strategies and evaluating therapeutic delivery across fetal membranes.

Background

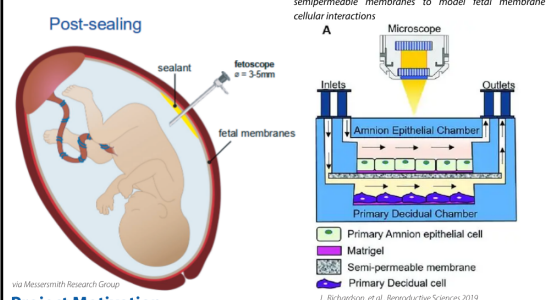
Fetal Membrane Injury in Fetal Surgery

Fetal surgeries performed during the late 2nd and early 3rd trimester are used to treat conditions such as spina bifida, congenital diaphragmatic hernia, and twin-twin transfusion syndrome. These procedures often require fetoscopic access that punctures the fetal membrane. Because fetal membranes exhibit limited regenerative healing, puncture defects can result in amniotic fluid leakage, preterm delivery (~30%), and increased maternal and fetal morbidity.

Need for Improved In Vitro Models

Recent studies have explored sealants and regenerative therapies for fetal membrane repair, including successful sealant studies in animal models [1]. Prior amnion-on-a-chip platforms demonstrated the feasibility of modeling fetal membrane cell interactions using compartmentalized microfluidic chambers separated by semi-permeable membranes [2]. However, existing in vitro amnion-on-a-chip systems often fail to recreate the native physical structure and barrier of fetal membranes or provide controlled quantification of puncture-induced leakage. This limits the ability to evaluate sealants and regenerative therapeutics under physiologically relevant conditions.

Figure 1. Clinical fetal membrane sealing strategies following fetoscopic puncture

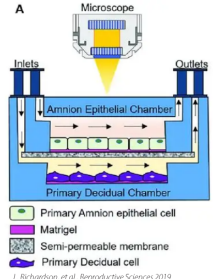


via Messersmith Research Group

Project Motivation

To address this gap, we developed an Amnion-on-a-chip microfluidic platform building upon these existing platforms, with the device designed to model membrane barrier integrity, simulate surgical puncture damage, quantify leakage testing, and enable future screening of sealants and regenerative therapeutics under controlled flow conditions.

Figure 2. Previously reported amnion-on-a-chip platforms use compartmentalized microfluidic chambers separated by semipermeable membranes to model fetal membrane cellular interactions



L. Richardson, et al., Reproductive Sciences, 2019

Methodology

Parameters:

Device size and geometry: 2 orthogonal PDMS microchannels, each with height x width of 60 μm x 180 μm

PCTE membrane: polycarbonate membrane sandwiched between 2 layers: pore size of 3 μm and a diameter of 13 mm

Centralized area: central puncture access region for simulated membrane injury/puncture

Post arrays: posts of diameter 800 μm (large) and 400 μm (small) to prevent PDMS roof collapse and to add additional support to our PCTE membrane

Time points: 5, 10, and 15 minutes, held at a constant flow rate of 1200 $\mu\text{L/hr}$ (time-varying analysis, for punctured v. unpunctured)

Flow rates: 600, 1200, and 2400 $\mu\text{L/hr}$ (constant collection intervals for punctured)

FITC concentration: 1 mM, diluted to 2.5 μM (50 μL 1 mM FITC: 20,000 μL DI) for plate reader

FITC excitation/emission wavelengths: 488 / 525 nm

Experimental Workflow & Quantification

Device Integrity Validation: To ensure leak-free bonding so that any observed transport is due to membrane behavior or fabrication defects.

Baseline (intact membrane) testing: To characterize the intrinsic barrier function of the membrane and establish a no-leakage baseline condition.

Flow-driven transport: To evaluate transport across the membrane under controlled flow conditions and establish baseline transport behavior.

Time-varying analysis: To assess whether transport remains stable over time and confirm the absence of spontaneous leakage (5, 10, 15 mins).

Flow rate variation: To assess whether changes in flow conditions induce transport, validating system sensitivity to pressure-driven effects (600, 1200, 2400 $\mu\text{L/hr}$).

Quantitative measurement (fluorescence): To obtain reproducible baseline measurements for future comparison with membrane disruption conditions (collection at lower-channel outflow).

Results & Analysis

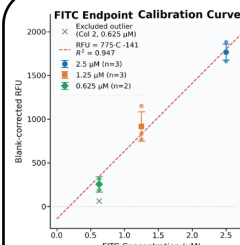


Figure 3. A FITC endpoint calibration curve was generated to convert plate reader fluorescence signals into estimated FITC concentrations. Blank-corrected RFU values increased linearly with FITC concentration, giving the calibration equation $\text{RFU} = 775.49 \times C - 140.90$ with $R^2 = 0.947$. One outlier at 0.625 μM was excluded because it deviated strongly from the expected linear trend. This calibration allowed the fluorescence measured from lower-channel outflow samples to be converted into FITC concentration for quantitative transport analysis.

Table 1. Back-calculated FITC concentrations were estimated from measured transported amount and collected volume for A) time-varying conditions, and from transport rate and flow rate for B) flow-varying conditions. While transport amount and rate increased with time and flow, respectively, the resulting effluent concentrations were variable, indicating diffusion-limited transport and highlighting that concentration is a less reliable metric than total transported FITC. The variability may reflect non-steady diffusion, sample collection differences, and limited replication.

A) Time-varying FITC Concentrations (@1200 $\mu\text{L/hr}$ 20 $\mu\text{L/min}$)					B) Flow-varying FITC Concentrations			
Time	Volume Collected (20 $\mu\text{L/min}$ * time)	FITC transported	FITC concentration (FITC transported / volume collected)		Flow Rate (per hr per min)	Transport rate	FITC concentration (transport rate / flow rate)	FITC amount (transport rate * 10 min)
5 min	100 μL	96.1 pmol	0.961 μM		600 $\mu\text{L/hr}$ ~ 10 $\mu\text{L/min}$	10.1 pmol/min	1.01 μM	101 pmol
10 min	200 μL	148.1 pmol	0.741 μM		1200 $\mu\text{L/hr}$ ~ 20 $\mu\text{L/min}$	15.8 pmol/min	0.790 μM	158 pmol
15 min	300 μL	258.6 pmol	0.862 μM		2400 $\mu\text{L/hr}$ ~ 40 $\mu\text{L/min}$	60.9 pmol/min	1.52 μM	609 pmol

Leakage Test

Device integrity was verified through a dual-validation approach. First, qualitative visual testing with a food dye tracer (image right) revealed a sharp boundary at the PDMS-PCTE interface. The absence of lateral seepage confirms that our bonding provides a robust, watertight seal under flow conditions. Second, this physical integrity is quantitatively supported by FITC transport data. If macroscopic structural leakage occurred, the bottom-chamber fluid would approach the highly concentrated 2.5 mM FITC input. Instead, collected samples consistently yielded trace amounts (pmol range). This steep concentration drop confirms that mass transport is strictly restricted to transmembrane diffusion, definitively ruling out device leakage.

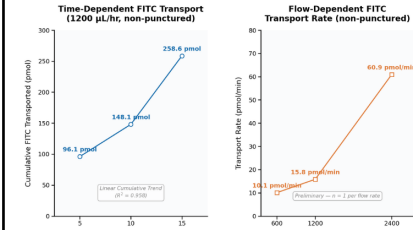


Figure 5. Time-dependent (A) and flow-dependent (B) FITC transport across a non-punctured PCTE membrane.

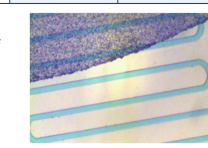


Figure 4. No observable lateral leakage at the PDMS-PCTE interface, confirming a robust seal under flow conditions

Baseline Transport Dynamics

To characterize the barrier function of the intact membrane, FITC transport was quantified across varying times and flow rates. Time-dependent analysis revealed a highly linear cumulative FITC transport. This constant flux confirms a stable, steady-state diffusion profile without structural degradation over the collection period. Flow-dependent analysis established the device's optimal operating window. Between 600 and 1200 $\mu\text{L/hr}$, transport rates increased moderately, characteristic of a stable diffusion-dominated regime. However, at 2400 $\mu\text{L/hr}$, the rate surged non-linearly to 60.9 pmol/min. This sharp deviation indicates that excessive flow induces high transmembrane pressure, leading to non-physiological convective bulk flow. Consequently, flow rates ≤ 1200 $\mu\text{L/hr}$ were identified as the functional upper limit to ensure pure diffusion-based transport, establishing a reliable baseline for evaluating future puncture-and-sealant models.

Device & Fabrication

CAD Design

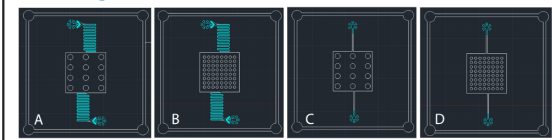


Figure 6. CAD designs of the four device variants. (A) Device with inlet/outlet resistors and filter, post diameter $d = 800$ μm . (B) Same configuration, $d = 400$ μm . (C) Device without resistors or filter, $d = 800$ μm . (D) Device without resistors or filter, $d = 400$ μm .

Device Features

Four device variants were fabricated to support two experimental objectives. Devices with resistors and filters (A, B) accommodate direct implantation of intact amniotic tissue onto the membrane prior to bonding. Devices without resistors or filters (C, D) allow cell seeding through the channel, as resistors would trap cells before reaching the membrane. Two post diameters (800 μm and 400 μm) were tested for structural support; both maintained chamber integrity without sagging. The 6 x 6 mm square chamber exceeds the 3 mm diameter of the smallest clinical fetoscopes, accommodating a realistic puncture while retaining structural integrity. Top and bottom channels are oriented orthogonally to prevent ports from overlapping on the device.

Fabrication Parameters (via SU-8 Datasheet)	
Target Thickness (μm)	80
Photoresist Type	SUB-2050
Spin Speed (RPM)	500 ~ 5 seconds 1800 ~ 30 seconds
Pre-exposure Bake	65°C; 3 minutes
Exposure Energy (mJ/cm^2)	215
UV Lamp Power (mW/cm^2)	10.25
Exposure Time (s)	20.98
Post-Exposure Bake	65°C; 2 minutes 95°C; 7 minutes

Table 2. SU-8 photolithography parameters used for mold fabrication, targeting a feature height of ~80 μm per layer.

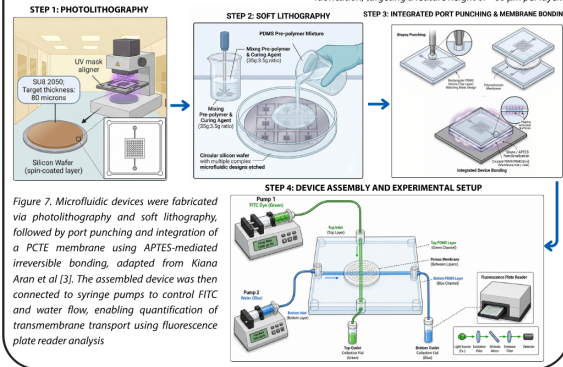


Figure 7. Microfluidic devices were fabricated via photolithography and soft lithography, followed by port punching and integration of a PCTE membrane using APTES-mediated irreversible bonding, adapted from Kiana Aran et al [3]. The assembled device was then connected to syringe pumps to control FITC and water flow, enabling quantification of transmembrane transport using fluorescence plate reader analysis

Discussion & Conclusion

Despite successful membrane puncture, no flow was observed in the bottom chamber, preventing us from collecting effluent for quantification. This may be due to insufficient pressure gradients, high hydraulic resistance in the lower channel, air bubble trapping, partial resealing (due to a tiny puncture), or blockage by PDMS/membrane debris.

Baseline data confirmed diffusion-dominated transport in the intact membrane, with increasing cumulative FITC over time and moderate scaling with flow at lower rates; deviation at higher flow suggests onset of convective effects. Back-calculated effluent concentrations were inconsistent, indicating that concentration is not a reliable primary metric in this system compared to total transported FITC and transport rate.

While post-puncture behavior could not be captured, we established a robust baseline model for intact membrane transport that can be extended to investigate puncture-induced leakage with improved device design. Future work will optimize priming, flow resistance, and puncture consistency for reliable post-puncture quantification.

References

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