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Mussel-mimetic tissue adhesive for fetal membrane repair: An ex vivo evaluation

C.M. Haller [a,f,1], W. Buerzle [b,1], A. Kivelio [a], M. Perrini [a], C.E. Brubaker [c], R.J. Gubeli [e], A.S. Mallik [a], W. Weber [e], P.B. Messersmith [c], E. Mazza [b,d], N. Ochsenbein-Koelble [a], R. Zimmermann [a], M. Ehrbar [a,f,*]

[a] Department of Obstetrics, University Hospital Zurich, Zurich, Switzerland
[b] Mechanical Engineering Department, Swiss Federal Institute of Technology, Zurich, Switzerland
[c] Biomedical Engineering Department, Northwestern University, Evanston, IL, USA
[d] Swiss Federal Laboratories for Materials Testing and Research, EMPA Dübendorf, Switzerland
[e] Faculty of Biology, BIOSS Centre for Biological Signalling Studies and SGBM Spemann Graduate School of Biology and Medicine, University of Freiburg, Germany
[f] Zurich Centre for Integrative Human Physiology, Switzerland

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ABSTRACT

Iatrogenic preterm prelabor rupture of membranes (iPPROM) remains the main complication after invasive interventions into the intrauterine cavity. Here, the proteolytic stability of mussel-mimetic tissue adhesive (mussel glue) and its sealing behavior on punctured fetal membranes are evaluated. The proteolytic degradation of mussel glue and fibrin glue were compared in vitro. Critical pressures of punctured and sealed fetal membranes were determined under close to physiological conditions using a custom-made inflation device. An inverse finite element procedure was applied to estimate mechanical parameters of mussel glue. Mussel glue was insensitive whereas fibrin glue was sensitive towards proteolytic degradation. Mussel glue sealed 3.7 mm fetal membrane defect up to 60 mbar (45 mm Hg) when applied under wet conditions, whereas fibrin glue needed dry membrane surfaces for reliable sealing. The mussel glue can be represented by a neo-Hookean material model with elastic coefficient C_1 = 9.63 kPa. Ex-vivo-tested mussel glue sealed fetal membranes and resisted pressures achieved during uterine contractions. Together with good stability in proteolytic environments, this makes mussel glue a promising sealing material for future applications.

1. Introduction

With advances in fetal diagnosis, the number of performed invasive interventions into the uterine cavity is increasing. Despite improvements in such procedures, even small-diameter fetoscopic access sites do not heal, and thus iatrogenic preterm prelabor rupture of fetal membranes (iPPROM) remains the main risk after fetoscopic procedures [1].

During the last decade several strategies to prevent iPPROM have been evaluated. Among such strategies were attempts to stimulate biological repair. Although some repair was observed in rabbit models [2,3], healing in sheep and rhesus monkeys was relatively limited [4,5]. It appears that dislocation of amnion and chorion as well as reattachment of fetal membranes (FMs) to the decidua rather than healing lead to sealing of the membrane defects [6]. Due to poor in vivo functionality and stability of materials, prophylactic plugging strategies have not advanced into clinical practice. Major limitations include lack of stable integration of scaffold materials, inability of biocompatible glues to bond to wet surfaces and susceptibility of naturally derived materials towards proteolytic degradation. Such shortcomings of materials generally lead to the instable plugging of the defect and subsequently to leakage shortly after application [7]. As FMs are temporary tissues and their repair mechanisms have recently been described as rather inefficient, the use of gluing materials that are not prone to in vivo remodelling might be envisaged, resulting in sealants that simply act as physical barriers to amniotic fluid.

Recently, star PEG-based polymers have been developed, which are either functionalized with the unusual amino acid 3,4 dihydroxyphenylalanine (DOPA) or catechol-presenting analogues thereof [8,9]. By conversion of catechol groups under oxidative conditions, highly reactive quinones are formed [10] that allow strong adherence of the polymer on wet surfaces and gel formation in a saline environment. A catechol functionalized PEG polymer mimic of mussel glue has been employed in a murine model of pancreatic islet transplantation in which absence of an inflammatory response, long term in vivo stability and good tissue integration were demonstrated [11]. In a recent in vitro study, the same mussel-mimetic tissue adhesive (“mussel glue”) has been described to be a non-cytotoxic sealant material that tightly adheres to FM [12].

* Corresponding author at: Department of Obstetrics, University Hospital Zurich, Zurich, Switzerland. Tel.: +41 44 255 8513; fax: +41 44 255 2889.
E-mail addresses: nicole.ochsenbein@usz.ch (N. Ochsenbein-Koelble), martin.ehrbar@usz.ch (M. Ehrbar).
[1] These authors contributed equally to this work.

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In order to test its mechanical properties, mussel glue was evaluated in a fully defined in vitro system using elastomeric membranes and a custom-made inflation device [13]. This study demonstrated that mussel glue can be distended and thus can adapt to shape changes of the underlying membranes. Although the ductility of the material appeared to be critical for the sealing of compliant membranes, membranes comparable in stiffness to FM were sealed efficiently. As the sealing of FM from such data cannot be extracted, the aim of this study was to evaluate the biochemical stability of mussel glue in vitro and its sealing properties under physiologically relevant conditions ex vivo.

2. Methods

2.1. Mussel glue

The production and characterization of mussel glue, a catechol-functionalized poly(ethylene glycol) (cPEG), which lacks the primary amine of the DOPA amino acid, was performed as described by Brubaker et al. [11]. For the formation of hydrogels, equal volumes of the cPEG precursor solution (300 mg ml^{-1} in phosphate-buffered saline (PBS)) and sodium periodate solution (12 mg ml^{-1} in water) were mixed and the reaction was allowed to cure for 5 min at room temperature.

2.2. Fibrin sealant

Clinical grade fibrin sealant (TISSEEL, Baxter AG, Vienna) was formed by mixing equal volumes of undiluted fibrin and thrombin solutions using the provided 2-component glue applicator "DUO Set" including the mixing device.

2.3. Plug stability

The polymerization of mussel glue and fibrin glue plugs of equal size was allowed to occur for 5 min under sterile conditions. The plugs were equilibrated overnight in cell culture medium (Dulbecco's modified Eagle medium (DMEM) Glutamax F12 + 1% penicillin/streptomycin) at 37 °C and 5% CO_2 before the weight of each plug was determined and set as 100%. The plugs were daily subjected to fresh cell culture medium with or without 8 mU plasmin (Sigma Aldrich GmbH, Switzerland) or 15 mU collagenase A (Roche, Switzerland). The weight of individual plugs was determined on a daily basis and used to determine the relative weight change.

2.4. Fetal membrane samples

FMs were collected from patients who underwent elective Caesarean section between 37 and 38 weeks of gestation. Patients were recruited for this study with informed written consent using a protocol approved by the Ethical Committee of the District of Zurich (study Stv22/2006). The pregnancies were randomly selected after thorough testing to exclude infections, e.g. HIV, hepatitis and streptococcus B. The selected pregnancies had no history of diabetes, connective tissue disorders and chromosomal abnormalities. After cutting the FM at least 2 cm away from the placental disc, the resulting membrane pieces were washed (PBS, pH 7.2, without Ca/Mg). Round membrane samples of ~7 cm diameter were cut out randomly and used within 4 h.

2.5. Design and setup of the inflation device

To assess the sealing properties of mussel mimetic sealant and fibrin sealant, a custom-made inflation device was employed that generated an equi-biaxial stress state in the central region of the circular samples. Membrane samples were mounted onto the fluid-filled aluminum cylinder with a 50 mm inner diameter and clamped by a cover ring which was designed to minimize the occurrence of membrane rupture at the sample periphery. The fluid pressure inside the cylinder was increased by a peristaltic pump (type 314VBM, four rollers, maximum 360 rpm, Watson-Marlow Ltd., Zurich, Switzerland), which is computer controlled and allows the inflation of the FM samples. The pressure was constantly recorded by a hydrostatic pressure sensor (Digital manometer, LEX 1, accuracy 0.05%, Keller, Switzerland). The inflation process was optically monitored by cameras (Point Grey, 1.4MP Color Grasshopper 1394b Camera, 2/3″ CCD) mounted on top and on the side of the cylinder.

2.6. Fetal membrane preparation, puncture and repair

FM samples were clamped between two sandpaper rings of 50 mm inner and 70 mm outer diameter to facilitate the mounting procedure. The intact FMs were placed such that the amnion was in contact with the fluid in the cylinder and the chorion on the outside. The cover ring was placed onto the water-filled cylinder of our loading device and fixed by a dynamometric screwdriver to reach an equal force of clamping at each screw.

Defects were created in the center of the mounted FM samples using a 16-gauge needle (1.6 mm diameter, Somatex) or a 11-French three-side pointed trocar of 3.7 mm diameter (Richard Wolf GmbH, Knittlingen, Germany). The resulting lesions were directly sealed with 125 μl mussel mimetic or fibrin sealant under wet conditions. For sealing experiments under dry conditions, samples were punctured, carefully dried and sealed before mounting them onto the water-filled cylinder.

2.7. Biaxial stretching of fetal membranes

The loading experiment was performed by inflating samples with a constant flow rate of 13.9 ml min^{-1} to continuously increase the pressure in the cylinder until rupture of the membrane sample. Rupture was characterized by a sudden decrease of internal pressure due to water leakage through a local lesion. The tissue deformation was tracked by the digital images from the side camera. The membrane profile extracted from each image provided information on the state of deformation associated with the corresponding value of internal pressure.

2.8. Histological evaluation of the membrane sealing

Intact and sealed tissue samples were embedded in paraffin and 4 μm cross-sections were cut using the rotation microtome (HM340E, Microtom GmbH, Walldorf, Germany). Hematoxylin and eosin (H&E) stained histological sections were analyzed with a Zeiss Axiovert 200 M microscope (Carl Zeiss, Switzerland).

2.9. Mechanical analysis of mussel glue

FMs are inhomogeneous and show a high inter- and intra-donor variability. For this reason, a mussel glue mechanical model was based on corresponding observations with inflation experiments on repaired elastomeric membranes [13].

An inverse finite element (FE) procedure was applied to estimate the mechanical parameters of mussel glue. The commercial finite element software package ABAQUS 6.6–3 was used to set up the corresponding axisymmetric model, consisting of the punctured membrane and the glue plug. Geometric and material nonlinearities were included. The elastomeric membrane had already been mechanically characterized [14]. The mussel glue was assumed to behave as a hyperelastic neo-Hookean material [15].