Acta Biomaterialia xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Engineered cell instructive matrices for fetal membrane healing

A. Kivelio [a,b], N. Ochsenbein-Koelble [a], R. Zimmermann [a], M. Ehrbar [a,c,*]

[a] Department of Obstetrics, University Hospital Zurich, Zurich, Switzerland
[b] Institute of Bioengineering, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland
[c] Zurich Centre for Integrative Human Physiology, Zurich, Switzerland

ARTICLE INFO

Article history:
Received 27 June 2014
Received in revised form 28 November 2014
Accepted 15 December 2014
Available online xxxx

Keywords:
Fetal membrane
Healing
Growth factor
PEG
Synthetic matrix

ABSTRACT

Iatrogenic preterm prelabour rupture of fetal membranes (iPPROM) occurs in 6–45% of the cases after fetoscopic procedures, posing a significant threat to fetal survival and well-being. The number of diagnostic and therapeutic prenatal interventions available is increasing, thus developing treatment options for iPPROM is becoming more important than ever before. Fetal membranes exhibit very restricted regeneration and little is known about factors which might modulate their healing potential, rendering various materials and strategies to seal or heal fetal membranes pursued over the past decades relatively fruitless. Additionally, biocompatible materials with tunable in vivo stability and mechanical and biological properties have not been available. Using poly(ethylene glycol)-based biomimetic matrices, we provide evidence that, upon presentation of appropriate biological cues in three dimensions, mesenchymal progenitor cells from the amnion can be mobilized, induced to proliferate and supported in maintaining their native extracellular matrix production, thus creating a suitable environment for healing to take place. These data suggest that engineering materials with defined mechanical and biochemical properties and the ability to present migration- and proliferation-inducing factors, such as platelet-derived growth factor, basic fibroblast growth factor or epidermal growth factor, could be key in resolving the clinical problem of iPPROM and allowing the field of fetal surgery to move forward.

1. Introduction

Iatrogenic preterm prelabour rupture of fetal membranes (iPPROM), which occurs in 6–45% of cases after fetoscopic interventions, is a serious complication associated with fetal morbidity and mortality [1]. It is linked with a range of serious complications, including respiratory distress syndrome, cerebral palsy, blindness, deafness and necrotizing enterocolitis [2]. After decades of research, iPPROM is still an unsolved clinical problem affecting millions of pregnancies worldwide. Compromising the expected benefits of any intrauterine intervention, it is a serious complication for prenatal diagnostics and fetal surgery, and a major obstacle for the further development of the field [3].

Human fetal membrane is a bilayer structure enclosing the amniotic cavity, consisting of a stronger inner layer, the amnion, and a more pliant outer layer, the chorion. Amnion is made of a stromal layer, a thick basement membrane and a single epithelium facing the amniotic cavity [4]. Because fetal membranes are very poorly vascularized, the lack of a typical wound-healing response, including inflammation, scar formation and tissue regeneration, as described in the skin [5] and many other organs, is not to be expected. As shown by clinical experience and animal studies, the healing potential of the membranes after fetoscopic intervention is very limited [6]. The lack of healing response is not completely understood, but could be explained by excessive matrix remodeling by matrix metalloproteinases (MMPs) [7], or most probably by the lack of a provisional cell-guiding matrix containing appropriate healing signals, normally provided by fibrin plugs.

Based on the hypothesis that cell-instructive scaffolds can induce a biological repair cascade, decellularized human amnion membranes, containing native matrix signals, have been evaluated both in vitro and in vivo in a rabbit mid-gestational model [8,9]. Porcine small intestine derived matrices [10], collagen or gelatin sponges [11–13], collagen slurry [14] and matrigel [13] have also been tested in in vivo models [8,15,16], with variable success. Fibrin sealants have been evaluated ex vivo, as well as in animal models and in patients [11,17]. Some of these repair strategies relying on naturally occurring materials have shown potential for iPPROM sealing, but, due to limited control over critical materials properties such as proteolytic stability and biological functionality, they could not be further optimized.

* Corresponding author at: Department of Obstetrics, PATH, G-48, Schmelzbergstr. 12, University Hospital, Zurich, Switzerland. Tel.: +41 44 255 85 13; fax: +41 44 255 28 89.
E-mail address: martin.ehrbar@usz.ch (M. Ehrbar).

http://dx.doi.org/10.1016/j.actbio.2014.12.011
1742-7061/

Fetal membranes have been hypothesized to harbor stem or progenitor cell populations as remnants of embryogenesis [18]. Several groups have investigated the potential of mesenchymal cells isolated from human amnion and have shown that these cells can differentiate into various lineages [19,20]. If amnion indeed contains a progenitor cell component, stimulating these cells with proper signals could initiate the healing of the punctured membranes. Thus, identification of materials and biological signals able to control and promote amnion cell recruitment, proliferation and extracellular matrix production would be of great clinical significance.

We have recently described modular designed by transglutaminase factor XIIIa (FXIII)-cross-linked poly(ethylene glycol) (PEG)-based biomimetic hydrogels [21,22] (named TG-PEG hydrogels) that are tailorable in terms of stiffness, proteolytic stability, and presentation of cell adhesion ligands and growth factors. These TG-PEG hydrogels, when formulated with low stiffness (30–250 Pa), MMP-degradable cross-links and 50 μM RGD, were shown to allow efficient migration of mesenchymal cells (osteoblasts and fibroblasts [23]). Additionally, the TG-PEG hydrogels permit the covalent immobilization of peptides, growth factors and other bioactive molecules that contained a short α2-plasmin inhibitor derived FXIII transglutaminase glutamine acceptor domain (Gln; NQEQVSPL) [22].

Taking advantage of these proteolytically stable, mesenchymal cell migration-compatible hydrogels, we herein describe the screening for soluble factors promoting and directing the healing of fetal membranes using in vitro and ex vivo models. We have established factors that can be employed for the in vitro mobilization of human amnion mesenchymal cells (hAMCs) from fetal membrane tissues and can support both their proliferation and the formation of cellular networks. This knowledge, together with available growth factor immobilization and release strategies [22,24], will provide the basis for the production of cell-instructive microenvironments for the healing of fetal membranes.

2. Materials and methods

2.1. Cell isolation, culture and characterization

Mesenchymal and epithelial cells were isolated from human amniotic membranes as described previously [18] and characterized using flow cytometry (a detailed description is provided in the Supplementary information). All experiments were repeated on cells from three different donors.

2.2. Formation of microtissues

Microtissues consisting of 1000 cells were formed by pipetting 30 μl drops of cells in culture medium containing 20% methylcellulose (Sigma Aldrich, St Louis, MO, USA) onto non-adhesive bacterial culture dishes, which were then turned upside down. Cells in the hanging drops were allowed to aggregate overnight at 37 °C, and the resulting microtissues were collected by washing the lid with 1% bovine serum albumin (BSA; Applichem) in phosphate-buffered saline (PBS). Microtissue suspension was centrifuged for 5 min at 56g and resuspended in cell culture medium.

2.3. PEG hydrogel formation

TG-PEG hydrogel formation was performed as previously described [21,22]. Briefly, functionalization of eight-arm PEG-vinylsulfone with the FXIII substrate peptides, namely a glutamine acceptor substrate or a lysine donor substrate containing an MMP-sensitive linker resulted in n-PEG-Gln or n-PEG-$MMP_{sensitive}$-Lys monomers [21,22]. Covalently cross-linked hydrogels were formed by adding 10 U ml^{-1} thrombin-activated factor XIIIa to a Tris-buffered saline (50 mM, pH 7.6) solution containing stoichiometrically balanced amounts of n-PEG-$MMP_{sensitive}$Lys and n-PEG-Gln, together with 50 mM calcium chloride and 50 μM Gln-RGD. Gelation occurred within a few minutes at room temperature, but the cross-linking reaction was allowed to further proceed for 30 min at 37 °C in a humidified incubator.

2.4. Cell, microtissue and tissue encapsulation into hydrogels

Suspension containing 6.5×10^4 cells ml^{-1}, microtissues or small pieces (0.5 × 0.5 mm) of homogenized tissue in culture medium were added to the gel mass immediately before FXIIIa. After the addition of FXIIIa, 20 μl gel drops were sandwiched between two hydrophobic glass slides separated by spacers (approximately 1 mm), which were manually rotated for the first 5 min of gel polymerization to avoid cell or tissue sedimentation into the bottom of the gel. After polymerization, gels were released and immersed in culture medium.

2.5. Analysis of 3-D cell migration

For migration studies, gels were glued to the bottom of cell culture wells with a 5% PEG gel formulation and equilibrated in culture medium for 2 h before starting the time-lapse acquisition. In order to study collective 3-D migration and screen for migration-inducing factors, microtissues were encapsulated in hydrogels. The chemotactic effects of several factors reported to act as chemoattractants to mesenchymal cells [25–29], including basic fibroblast growth factor (bFGF; Peprotech, Rocky Hill, NJ, USA), epidermal growth factor (EGF; Sigma, St Louis, MO, USA), platelet-derived growth factor (PDGF; Peprotech), transforming growth factor beta (TGF-β; Peprotech), tumour necrosis factor alpha (TNF-α; Biosource, Camerillo, CA, USA), insulin-like growth factor two (IGF-2, Peprotech), stromal-derived factor one (Peprotech), interleukin-6 (R&D Systems, Minneapolis, MN, USA), vascular endothelial growth factor (Peprotech) and hepatocyte growth factor (Peprotech), were tested by adding them individually or in combinations into the medium (all 100 ng ml^{-1}).

In experiments directed towards studying growth factor-induced migration mechanisms, the broad-spectrum MMP inhibitor GM6001 and the Rho associated protein kinase (ROCK) inhibitor Y-27632 (both from Calbiochem) were added 1 h prior to the addition of the growth factors. The inhibitors were used at concentrations of 50 μM (GM6001) and 10 μM (Y-27632), as recommended by the manufacturer and described in the literature [23].

Migration was visualized using time-lapse microscopy (images captured every 10 min up to 48 h). Collective migration out of microtissues was quantified by determining the area covered by the migrated cells from still images after 48 h and 5 days using ImageJ [30]. The mean migration speed of single dispersed encapsulated cells was determined from the resulting movies using the MOSAIC particle tracker plug-in for ImageJ [31].

2.6. Analysis of cell viability, metabolic activity and proliferation

Cell viability in 3-D hydrogel cultures after 1 week of growth factor stimulation was examined using a LIVE/DEAD® Viability/Cytotoxicity Kit (Molecular Probes, Eugene, OR, USA). Briefly, ethidium homodimer-1 and calcein AM from the kit were diluted in a 1:1000 ratio in culture medium and gels were stained with this solution for 10 min in a cell culture incubator before imaging.

The DNA content was measured using a CyQUANT® NF Cell Proliferation Assay Kit (Invitrogen). After culture periods of 0, 2, 5 and 10 days, the gels were rinsed with PBS and stored at −80 °C until

Please cite this article in press as: Kivelio A et al. Engineered cell instructive matrices for fetal membrane healing. Acta Biomater (2015), http://dx.doi.org/10.1016/j.actbio.2014.12.011