Placenta xxx (2017) 1–7

Contents lists available at ScienceDirect

Placenta

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

The application of decellularized human term fetal membranes in tissue engineering and regenerative medicine (TERM)

Aida Shakouri-Motlagh [a, b, 1], Ramin Khanabdali [b, c, 1], Daniel E. Heath [a, **], Bill Kalionis [b, c, *]

[a] Department of Chemical and Biomolecular Engineering, Particulate Fluids Processing Centre, The University of Melbourne, Parkville, Victoria, Australia
[b] Department of Maternal-Fetal Medicine, Pregnancy Research Centre, Royal Women's Hospital, Parkville, Victoria, Australia
[c] Department of Obstetrics and Gynaecology, University of Melbourne, Parkville, Victoria, Australia

ARTICLE INFO

Article history:
Received 12 February 2017
Received in revised form
30 June 2017
Accepted 3 July 2017

Keywords:
Fetal membranes
Extracellular matrix
Stem cells
Tissue engineering
Decellularized tissue

ABSTRACT

Tissue engineering and regenerative medicine (TERM) is a field that applies biology and engineering principles to "restore, maintain or repair a tissue after injury". Besides the potential to treat various diseases, these endeavours increase our understanding of fundamental cell biology. Although TERM has progressed rapidly, engineering a whole organ is still beyond our skills, primarily due to the complexity of tissues. Material science and current manufacturing methods are not capable of mimicking this complexity. Therefore, many researchers explore the use of naturally derived materials that maintain important biochemical, structural and mechanical properties of tissues. Consequently, employing non-cellular components of tissues, particularly the extracellular matrix, has emerged as an alternative to synthetic materials. Because of their complexity, decellularized tissues are not as well defined as synthetic materials but they provide cells with a microenvironment that resembles their natural niche.

Decellularized tissues are produced from a variety of sources, among which the fetal membranes are excellent candidates since their supply is virtually unlimited, they are readily accessible with minimum ethical concerns and are often discarded as a biological waste. In this review, we will discuss various applications of decellularized fetal membranes as substrates for the expansion of stem cells, their use as two and three-dimensional scaffolds for tissue regeneration, and their use as cell delivery systems. We conclude that fetal membranes have great potential for use in TERM.

1. Fetal membranes

The human fetal membranes envelop the fetus and amniotic fluid to form a sac, which acts as an interface with the mother and protects the fetus during pregnancy [1,2]. In the following sections, we summarize important aspects of the structure and properties of human fetal membranes, the various stem cell populations harboured in human fetal membranes, and briefly describe the history of use of fetal membranes as biomaterials.

1.1. Structure and properties of term fetal membranes

Fetal membranes are comprised of two components that can readily be separated; the amnion and chorion. The amnion is an avascular tissue that is most proximal to the fetus, comprising a single layer of amniotic epithelium, which is in direct contact with amniotic fluid [1,3] and an underlying stroma layer (Fig. 1) [4].

The chorion surrounds the amnion and consists of four layers that are represented in Fig. 1. Underlying the chorionic stroma is the firmly attached maternal *decidua* [1]. For this review, the chorionic stroma and attached *decidua* are referred to as the choriodecidua. Thus the fetal membranes are complex, multilayered structures of fetal origin that are firmly attached to maternal *decidua*.

1.2. Stem cells in fetal membranes

Fetal membranes harbor various relatively unexploited stem/progenitor cell types. Fetal membrane-derived stem/progenitor

* Corresponding author. Department of Maternal-Fetal Medicine, Pregnancy Research Centre, Royal Women's Hospital, Parkville, Victoria, Australia.
** Corresponding author.
E-mail addresses: daniel.heath@unimelb.edu.au (D.E. Heath), bill.kalionis@thewomens.org.au (B. Kalionis).
[1] Authors with the same contribution to this paper.

http://dx.doi.org/10.1016/j.placenta.2017.07.002
0143-4004/

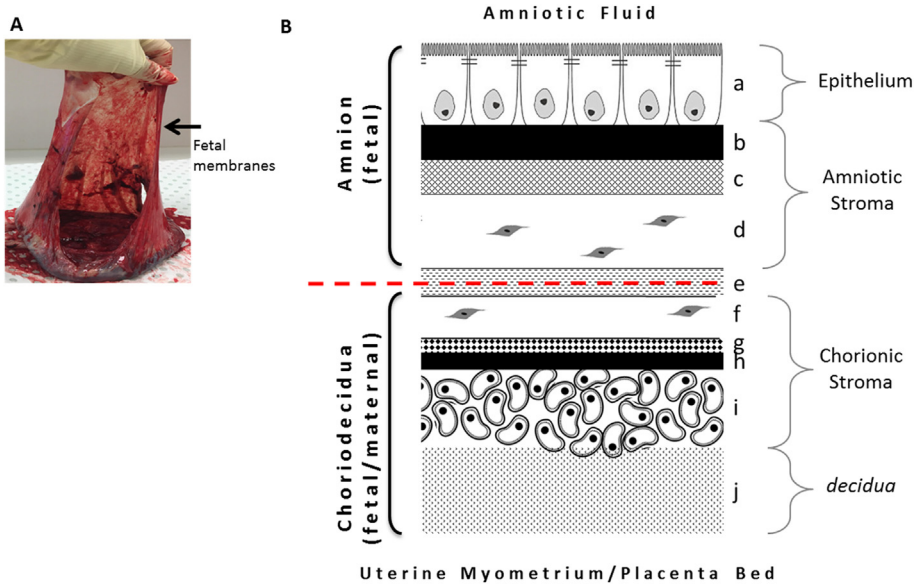

Fig. 1. Schematic depiction of fetal membranes and different sections.
(A) A placenta with the fetal side facing up and the umbilical cord attached. Fetal membranes are a thin flat sheet attached to the placenta bed on one side and form a sac to surround the fetus and amniotic fluid on the other side. (B) Fetal membranes comprise two membranes; amnion and chorion. Amnion consists of four different layers, a layer of epithelium cells (a), attached to an underlying basement membrane (b), a dense compact layer underneath (c), and finally a layer of dispersed fibroblasts (d). Chorion consists of four layers, a narrow fibroblast layer (f), a reticular layer (g), a pseudo-basement membrane (h) and a layer of cytotrophoblast cells of varying thickness (i) with an underlying *decidua* (j). Dash line showed the part where amnion and chorion can be split apart (e).

cells include human amniotic epithelial cells (hAECs), human amniotic mesenchymal stem cells (hAMSCs), human chorionic mesenchymal stem cells (hCMSCs), and *decidua parietalis* mesenchymal stem cells (DPMSCs) [5,6]. Fetal membranes-derived stem/progenitor cells have distinctive properties that differentiate them from many other sources. First, they originate at early stages of embryonic development, and maintain their pluripotent characteristics even when they are isolated from term placentae [7]. Second, these stem cells are present in different microenvironments (i.e. niches) in fetal membranes, which include both perivascular and avascular niches [8]. Third, DPMSCs are of maternal origin, whereas all other stem/progenitor cells in fetal membranes are of fetal origin. Finally, stem/progenitor cells from fetal membranes (and other placenta and birth-associated tissues) have advantageous immunomodulatory properties compared with other sources of MSCs [9]. The properties of fetal membrane-derived stem/progenitor cells are comprehensively reviewed elsewhere [1,5,7,10].

The different mesenchymal stem cell (MSC) niches in fetal membranes are of particular interest because studies show that expanding MSCs on decellularized ECM (dECM) derived from bone marrow MSCs, affects a wide variety of MSC properties including cell adhesion, proliferation, differentiation and expression of stemness markers [11]. In contrast, MSCs grown on dECM derived from non-stem cell sources such as human neonatal dermal fibroblast, or on extracellular matrix from mouse sarcoma tissue (Matrigel®), did not show similar effects [12,13]. Fetal membranes have distinct advantages over bone marrow MSCs as sources of dECM in terms access, abundance and yield of MSCs. However, equally important is that MSCs from different fetal membrane niches (i.e. maternal and fetal, vascular and avascular) have been shown to produce dECMs that support MSC expansion in different and potentially beneficial ways to that of dECM derived from bone marrow [14].

1.3. Fetal membranes as biomaterials

Fetal membranes are promising biomaterials because they are inexpensive, are readily available [15], and the supply is virtually unlimited with an annual global production of 15 million square metres per year [16]. Other desirable properties of fetal membranes for regenerative medicine applications include their minimum inflammatory responses and scar formation [2,3], biostability, vasoactivity, thromboresistance [17] and antibacterial properties [3].

Fetal membranes, predominantly the human amnion (AM), have been used as a biomaterial for over 100 years. The first application for fetal membranes was a skin replacement for burn and ulcer injuries in the early 20th century [4,18]. Since then, fetal membranes have been used to treat different skin pathologies (including burns and diabetic and bedsore ulcers) with improved re-epithelization as well as reduced pain and scarring [2,19]. Fetal membranes were also used to treat various ocular pathologies including chemical burns [19,20], scleral thinning and bullous keratopathy [21], for vaginal reconstruction in cases of agenesis or after vaginectomy, as a graft in congenital absence of the vagina [22], and for dental defects [21] Other uses include cartilage repair [23], as a physical barrier to replace defective peritoneum, and for the prevention of adhesion of intra-abdominal organs to the uterus and pelvis [24]. More recently they were used as scaffolds for peripheral nerve regeneration [25].

When intact membranes are implanted in the majority of tissues, they induce an immune response [26,27]. As an example, when intact fetal membranes were implanted in mouse, a fibrous capsule surrounds the host tissue that is an indicative of an immune response [26]. This adverse immune response necessitated decellularization of the membranes. Indeed, the non-cellular components of tissues are well tolerated, even when used as a xenograft [28]. When decellularized membranes are used, the thickness of the fibrous capsule reduced significantly and the host tissue showed significantly more integration of the implanted membrane [26]. Therefore, decellularization of the tissue is an appealing strategy for minimising inflammatory responses.

Moreover, by removing cells, the extracellular matrix (ECM) components are more exposed [29], which promotes tighter cell-ECM interactions and results in more efficient cell attachment

Please cite this article in press as: A. Shakouri-Motlagh, et al., The application of decellularized human term fetal membranes in tissue engineering and regenerative medicine (TERM), Placenta (2017), http://dx.doi.org/10.1016/j.placenta.2017.07.002