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Short communication

Different metabolic activity in placental and reflected regions of the human amniotic membrane

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ABSTRACT

Cells of the human amniotic membrane (hAM) have stem cell characteristics with low immunogenicity and anti-inflammatory properties. While hAM is an excellent source for tissue engineering, so far, its sub-regions have not been taken into account. We show that placental and reflected hAM differ distinctly in morphology and functional activity, as the placental region has significantly higher mitochondrial activity, however significantly less reactive oxygen species. Since mitochondria may participate in processes such as cell rescue, we speculate that amniotic sub-regions may have different potential for tissue regeneration, which may be crucial for clinical applications.

1. Introduction

The human amniotic membrane (hAM) is the innermost of the fetal membranes. Devitalized, it has already been applied in clinics for decades for wound dressing or cornea replacement. The hAM contains epithelial and mesenchymal cells with proven stem cell properties [1,2], and vital hAM can differentiate into lineages such as osteogenic [3], chondrogenic [4], and Schwann cell-like [5]. Moreover, hAM is immunomodulatory [2], anti-inflammatory, anti-fibrotic, anti-microbial and non-tumorigenic [6]. This, together with its unique architecture, makes hAM a promising material for tissue regeneration.

For clinical applications of a vital biomaterial, profound knowledge of cell identity and characteristics including differentiation capacity and quality is a prerequisite. Cell differentiation is highly energy-consuming and mitochondrial activity is closely related to cell type and function. Several groups demonstrated mechanisms of mitochondrial transfer to rescue injured cells, *in vitro* and *in vivo* [7–11]. Thus, higher mitochondrial activity might impact tissue repair.

Biomaterials can vary strongly in quality. To ensure consistent quality for therapeutic purposes, factors such as donor age, isolation method and site of withdrawal may need consideration. The hAM can be partitioned in placental (PA) and reflected amnion (RA), and differential gene expression has already been shown by Han *et al.* in these sub-regions, partly depending on the onset of labour [12]. Sub-regional differences in post-transcriptional regulation of gene expression by microRNA, important at parturition, where described by Kim *et al.* [13].

Scope of this study was to investigate regional differences of mitochondrial distribution, quality and activity in cells of hAM.

2. Material and methods

2.1. Preparation of hAM

Human placentae were collected after caesarean sections with informed consent and local ethical board approval. PA and RA were

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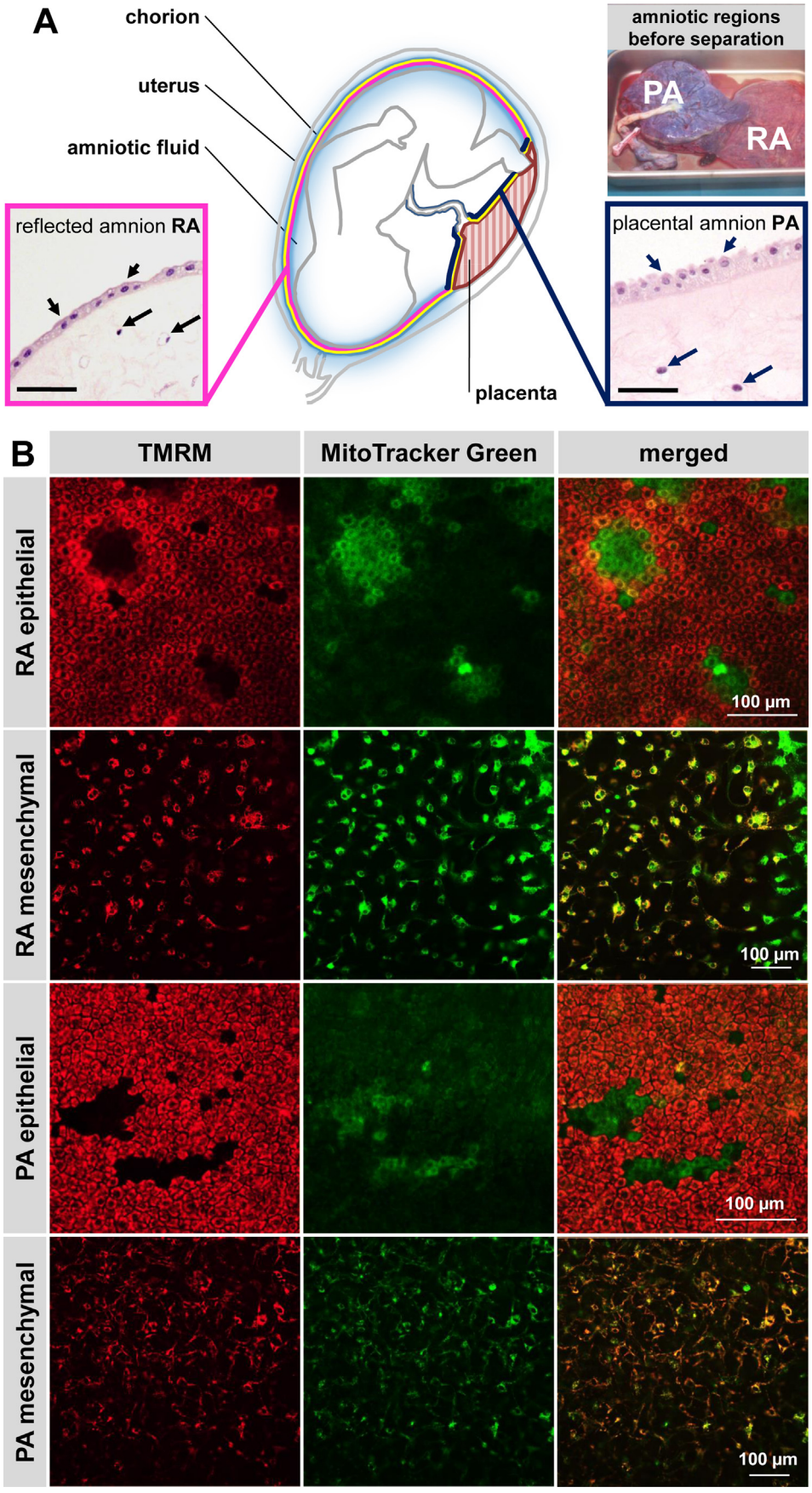

Fig. 1. A. Topology of human amniotic membrane. Uterus (grey), placenta (red), chorion (yellow), placental amnion PA (dark blue), reflected amnion RA (magenta). Haematoxylin/eosin staining of histological sections. Epithelial cells of PA are cylindrical with decentralized nuclei, epithelial cells of RA are flatter and more homogenous. Long arrows indicate mesenchymal cells, short arrows epithelial cells. Scale bars 100 µm. B. Representative images of LSM analysis. Biopsy punches of human amniotic membrane (4 donors) of 26 mm were mounted on cell crowns on day 2 and stained with 500 nM TMRM (red) and 250 nM MitoTracker Green FM (MTG, green). Epithelial and mesenchymal cells of both regions are heterogenous regarding their mitochondrial membrane potential.