SECOND HARMONIC IMAGING OF HUMAN FOETAL MEMBRANE: NORMAL AND ALTERED MORPHOLOGY

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Introduction

Characterizing the mechanical behaviour of the foetal membrane (FM) is important towards prediction and prevention of their spontaneous or iatrogenic (foetal surgery, amniocentesis) premature rupture [Beck, 2011; Calvin, 2007]. The zone of altered morphology (ZAM) identified over the cervix [McLaren, 2000] is suspected of being mechanically weak and thus the origin of FM rupture [El Khwad, 2005]. In this study, for the first time the second harmonic generation technique [Cox, 2011] was combined with an in-situ stretching device in order to investigate the layered structure of FM at different levels of deformation, and to visualize its alteration in ZAM and after cyclic mechanical loading.

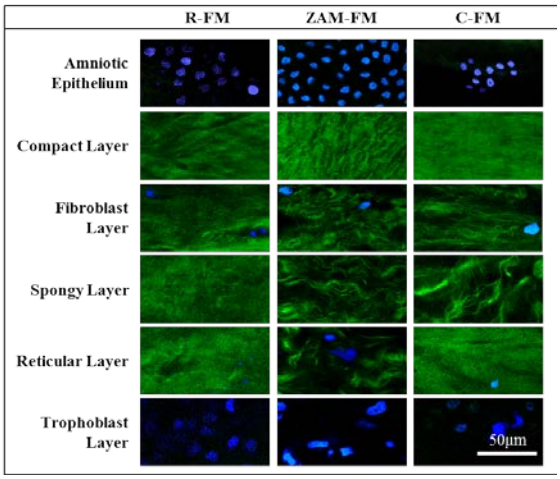

Figure 1: Second harmonic imaging of reflected (R-FM), zone of altered morphology (ZAM-FM) and cycled (C-FM) samples.

Methods

Five FMs were marked over the cervix during caesarean sections. Samples of ZAM and reflected tissue (harvested between the placental border and ZAM) were stained with DAPI and mounted in the stretching device into the microscope. From each membrane, one sample of reflected tissue was cyclically stretched using an inflation device [Perrini, 2012] before microscopy. Specimens were imaged through the thickness every 3 µm at 0%, 20% and 40% of strain and the orientation of collagen structures quantitatively analysed.

Results

The cellular (nuclei in blue) and collagen (in green) structures allow to identify the characteristic layers of FM (Fig. 1). In the fibroblast and spongy layers of ZAM-FM and C-FM, collagen consistently displays a non-uniform structure with curly fibre bundles, as opposed to a homogeneous appearance of R-FM. The reticular layer appears similarly altered in ZAM-FM, but not in C-FM. Collagen was observed to align in loading direction, forming bundles of oriented fibres (Fig. 2).

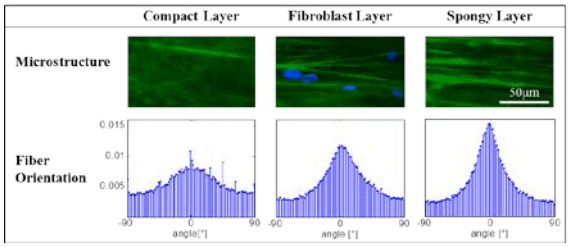

Figure 2: Examples of orientation at 40% stretch for different layers.

Discussion

Second harmonic imaging was shown to provide relevant information on FM microstructure and its deformation behaviour. Microstructural parameters were obtained, e.g. thickness, collagen orientation and local deformation, which can be used to formulate and verify FM constitutive models. Alteration of the spongy and fibroblast layers in C-FM is similar to the effects observed in ZAM-FM. This might indicate that the interface between amnion and chorion in the ZAM is affected by mechanical loading in addition to biochemical factors [McLaren, 2000].

References

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