HYPOXIA AS A STIMULUS UPON NEONATAL SWINE MENISCUS CELLS: HIGHWAY TO PHENOTYPIC MATURATION OF MENISCAL FIBRO-CHONDROCYTES?

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Menisci are essential structures in the knee joint where they cover fundamental biomechanical and protective roles (1, 2, 3). Menisci are characterized by a peculiar structure that, on one hand, allow them to perform their particular role in the stifle joint, but simultaneously make them a very challenging structure to deal with (2). Immature menisci are featured by numerously elongated cells (fibrocytes-like) in a disorganized matrix composed almost completely of collagen type I and few glycosaminoglycans (GAGs) and have a rich vascularization, on the other hand, mature and functional menisci are characterized by few round-shaped cells, a matrix rich of well ordinated collagen fibers (above all collagen type II) and GAGs, and preserve vascularization only in the outer zone (aka *red zone*) (1). Great interest, in both human and veterinary medicines, is reserved to the treatment of the injuries of the inner and avascular zone (aka *white zone*) of the meniscus: until now, there are no perfect solutions for the regeneration or the replacement of this tissue once injured (3).
This work is focused on the utilization of an environmental factor like hypoxia in meniscal tissue culture, in order to evaluate if it could be utilized to improve meniscal culture with a view to tissue engineering. Ninety menisci from neonatal pigs (day 0) were harvested and cultured under two different atmospheric conditions (hypoxia with 1% O_2 and normoxia) until 14 days. Samples were analysed at 0, 7 and 14 days through histochemical (Safranin-O staining), immunofluorescence and RT-PCR (Sox-9, Hif-1a, Hif-2, Collagen I and II, both methods) and biochemical (DNA, GAGs, DNA/GAGs ratio) techniques to record any possible differences in maturation of meniscal cells. Safranin-O staining allowed to show an increment in matrix deposition and round-shape "fibro-chondrocytic" cells quantity of hypoxia-cultured menisci respect to controls under normal atmospheric conditions. The same maturation shifting was observed by means of immunofluorescence and RT-PCR analysis, characterized by an increment of Sox-9 and collagen II, moving from day zero to 14-days under hypoxic environment, and by biochemical analysis, with an increment of DNA/GAGs ratio typical of mature meniscal tissue (characterized by few cells and much GAGs). This study shows that hypoxia can be considered as a booster to achieve meniscal cells maturation and opens considerably opportunities in the field of meniscus tissue engineering.

References:

1. Dai Z. et al. J Orthop Res. 2013, 31:1514–1519,

2. Fox AJS et al. Clin Anat. 2015, 28:269–287

3. Sosio C. et al. Tissue Eng Part A. 2015, 21:3-4.