miRNAMap and *miRGen*) combining gene expression information with miRNA target prediction.
Results: We identified 7 up-regulated miRNA in both normal and OA chondrocytes (miR-663, miR-638, miR-125b, miR-23a, miR-23b, miR-103, miR-210) that through bioinformatic analysis could be related with the chondrocyte phenotype and with the hypoxia condition of the aggregate culture. In addition, 4 miRNA were up-regulated in normal chondrocytes (miR-18a, miR-801, miR-370, miR-224) and related with growth factors. Finally, 5 miRNA were up-regulated in OA chondrocytes (miR-656, miR-138, miR-369-3p, miR-143, miR-181d), related with inflammatory pathways and transcription factors implicated in the regulation of chondrogenesis.
Conclusions: The data reported can be relevant to better understand the molecular mechanism involved in the biology and in the pathophysiology of the chondrocytes. This preliminary study could help to improve the understanding of the pathogenesis of multifactorial diseases such as osteoarthritis and could have important diagnostic and therapeutic potential.
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173

MULTILINEAGE DIFFERENTIATION POTENTIAL OF HUMAN AMNIOTIC MEMBRANE-DERIVED STEM CELLS IS USEFUL FOR HUMAN ARTICULAR CARTILAGE REPAIR

E. Muiños[1], S. Diaz-Prado[1,2], C. Cicione[1], E. Rendal[3], I.M. Fuentes[1,2], F.J. De Toro[1,2], **F.J. Blanco**[1]
[1]Osteoarticular and Aging Res. Lab., CIBER-BBN, Rheumatology Div., INIBIC-Complejo Hosp. Univ. A Coruña, A Coruña, Spain; [2]INIBIC-Univ. of A Coruña, A Coruña, Spain; [3]Criobiology Unit, CIBER-BBN, INIBIC-Complejo Hosp. Univ. A Coruña, A Coruña, Spain

Purpose: Amniotic membranes have been used extensively as biologic dressings in ophthalmic, abdominal, and plastic surgery. Actually, there is growing evidence that the human amnion contains various types of stem cell. Human amniotic membrane (HAM) could be an alternative source to bone marrow. As amniotic membrane is highly abundant and readily available, it has the potential to be an important source of mesenchymal stem cells (MSCs) with multilineage differentiation potential for human articular cartilage repair. Given the minimal ethical and legal issues associated with its usage warrants further investigation into their functional potential in vivo. The aim of thisstudy was investigate the potential of mesenchymal cells derived from HAM to differentiate into chondrocytes, adipocytes and osteoblasts useful for regenerative medicine and cell therapy.
Methods: Human amniotic membranes were obtained from caesarean-sectioned mothers. Two different protocols of HAM isolation were performed. After monolayer expansion of the adherent cells, isolated from both protocols, we characterized them by flow citometry for MSC markers. Multipotentiality was studied by means of the differentiation towards adipocytes, osteoblasts and chondrocytes-like cells. Adipogenesis and osteogenesis were induced using commercial medium (Lonza Group Ltd, Switzerland). Chondrogenesis was performed using cells in aggregate culture and in a chondrogenic medium for 3 weeks. Histochemical (hematoxylin-eosin, Masson's thrichome, toluidine blue, safranin O, Oil-Red-O, Alizarin Red), immunohistochemical (type II and I collagens, aggrecan) and qPCR studies were performed in order to evaluate the multilineage potential. For qPCR analysis the following primers were used: Sox9, Agg and Col II (for chondrogenesis), LPL, FABP4 and AMP1 (for adipogenesis), ALP and OC (for osteogenesis).
Results: From each of the protocols we isolated two different populations of mesenchymal cells derived from HAM. Both populations were characterized by the presence of the same cell surface markers with the exception of CD44, CD73, CD105, CD166 and CD117 markers. However, only one of the two populations had a strong capacity to differentiate towards chondrocytes, adipocytes and osteoblast. In this population, after 21 days of culture, the differentiations showed to be consistent and were confirmed by qPCR analysis.
Conclusions: We isolated a high pluripotency mesenchymal stem cell population from HAM, suggesting that they may be very useful to repair articular cartilage injuries and to improve the understanding of the molecular events implicated in chondrogenesis. Further studies should be carried out to determine whether such *in vitro*-differentiated cells can function *in vivo*.
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174

THE EFFECT OF LOW-INTENSITY PULSED ULTRASOUND FOR SACFFOLD-FREE CHONDROCYTE PLATE IN VITRO AND IN VIVO

K. Uenaka, S. Imai, S. Shioji, K. Kumagai, N. Okumura, Y. Matsusue
Shiga Univ. of Med. Sci., Otu,Shiga, Japan

Purpose: The aim of this study is to evaluate the effect of low-intensity pulsed ultrasound (LIPUS) for scaffold-free chondrocyte plate in vitro and in vivo.
Methods: Chondrocytes were collected from articular cartilage of Japanese white rabbits. For acquirement the number of cells, the collected primary chondrocytes (passage 0, P0) were cultured up to subconfluent in 500cm^2 square dishes. The cells were then condensed to the density at 10^7cells/cm^2 (passage 1, P1) on synthetic membranes with 0.2μm pore. The LIPUS application group was stimulated for 20 min/day. The mode of the applied ultrasound is a 200μs burst sine wave of 1.5 MHz repeating at 1kHz with an intensity of 30mW/cm^2. To investigate effect LIPUS stimulation on the matrix-synthesis of the constructs, mRNA expression of type II collagen (col2), aggrecan and typeIcollagen (col1) was studied using real-time polymerase chain reaction. Synthesis of type II collagen and proteoglycan was also assessed histochemically. We made full-thickness cartilage defect model in rabbit and tried to repair full thickness cartilage defect with allograft of the chondrocyte plate, and assessed histochemically.
Results: In our previous study, we presented that high-density culture with P1 chondrocytes more than 10^7cells/cm^2 could form a cell mass as scaffold-free cartilage under existing cell-cell interactions in rat model.
In this way, the chondrocytes (P1) prepared at 10^7cells/cm^2 detached from the membranes to form a plate of chondrocytes around the 7th day (day 7) of starting P1 culture. After forming the plate, the constructs were detached then by surgical pincers and moved to simple 6 well dishes for oxygen and nutrition diffusion. The expression of col2 and aggrecan mRNA was significantly higher in the group by stimulation of LIPUS (LIPUS group) than the group by no stimulation (sham group).