An Innovative Laboratory Procedure to Expand Chondrocytes with Reduced Dedifferentiation

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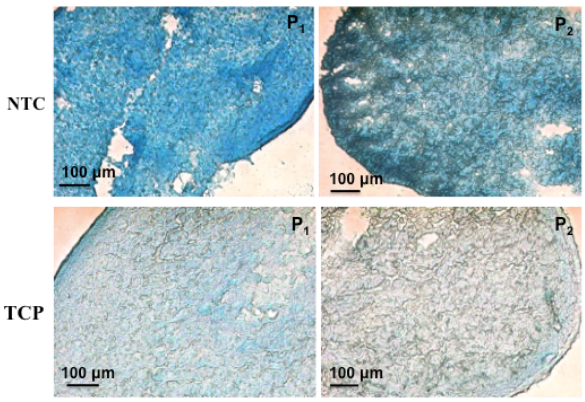
Statement of Purpose: In vitro culture of chondrocytes is required for cartilage tissue engineering and clinical practices[1]. However, the challenge is that the dedifferentiation of chondrocytes occurs during in vitro expansion[2,3]. Dedifferentiation of the chondrocytes results to the lose of their characteristics and the adaption fibroblast-like phenotypes. This study focuses on identifying an optimal cell culture surface to reduce the dedifferentiation while supporting chondrocyte expansion in vitro.

Methods: Freshly isolated primary bovine chondrocytes were cultured on conventional tissue culture treated polystyrene surface (TCP) and on a non-tissue culture treated surface (NTC) for multiple passages. For each passage, the cell numbers were counted and expressions of chondrocyte markers were monitored by qPCR and sGAG quantification. The chondrocytes expanded on TCP or NTC were evaluated in a pellet culture for 3 weeks and the production of chondrocyte extracellular matrix in the pellets was quantified. The chondrocytes expanded on TCP or NTC were also evaluated in a nude mice subcutaneous impantation model. After 6 weeks, the explants were analyzed by histology.

Results: Comparing with the conventional tissue culture treated polystyrene surface (TCP), a non-tissue culture treated surface (NTC) supported bovine chondrocyte proliferation to a clinically relevant expansion requirement within 2 passages. Additionally, chondrocytes cultured on NTC better maintained the chondrocyte phenotype compared to cells cultured on TCP in a standard medium (DMEM plus 10% fetal bovine serum). A pellet culture study was carried out and showed that chondrocytes expanded on NTC expressed higher level of chondrocyte extracellular matrix. The pellet culture seemed to reverse the dedifferentiation that occurred on NTC but could not reverse the dedifferentiation that occurred on TCP. The cells expanded on NTC or TCP were implanted subcutaneously as pellets to nude mice. After 6 weeks, the recovered pellets showed cartilage-like tissue formation from cells expanded on NTC but not from the cells expanded on TCP.



Alcian blue staining of pellets cultured from chondrocytes expanded on NTC or TCP after one passage (P1) or two passages (P2)

Conclusions: In this study, we presented an innovative culturing procedure to expand chondrocytes in vitro with reduced dedifferentiation. This procedure has a potential to be developed to expand chondrocytes in vitro for basic research, cartilage tissue engineering even clinical applications.

References:

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3. Schnabel M, Marlovits S, Eckhoff G, Fichtel I, Gotzen L, Vecsei V, et al. Dedifferentiation-associated changes in morphology and gene expression in primary human articular chondrocytes in cell culture. Osteoarthritis Cartilage 2002;10:62-70.