RELEVANCE: Propagation of chondrocytes is a very important step before in vitro cell seeding or in vivo transplantation for repairing cartilage defect.

INTRODUCTION: For cartilage repair, chondrocytes have been isolated from articular cartilage, periosteum, or growth plates, duplicated in culture, and transplanted into articular defects, secured by a piece of periosteum. A newer approach is using chondrocyte seeded scaffold for cartilage repair with results. However, no matter what method is used, chondrocytes need to be propagated in vitro before any transplantation or cell seeding.

Agarose culture is a method to maintain chondrocyte phenotype. But, the cells in agarose are not readily collectable because of the difficulty in dissolving the agarose gel by agarase. A thermosensitive polymer, a copolymer of poly(N-isopropylacrylamide) and acrylic acid (PNIPAAm-co-Aac), has the ability of gelling at 37°C, the lower critical solution temperature (LCST) and liquidizing under it (1). The new copolymer has been used as cell matrix in the production of a biohybrid artificial pancreas. In our laboratory, fibroblasts have also survived in the polymer gel and no toxicity has been found.

The hypothesis of this study was that chondrocytes could 1) duplicate in the copolymer gel, 2) retain their chondrocyte phenotype, and could 3) be easily recovered from the gel by simply lowering the temperature below LCST.

To verify the hypothesis, chondrocytes from adult rabbit scapular cartilage were harvested and cultured in a monolayer culture until confluency (~2 wks). Then, the cells were harvested and seeded into the copolymer gel and cultured for 2-3 wks. The phenotype of the cultured cells was then characterized. Two groups of control cultures, monolayer and agarose gel, were used to compare their ability of maintaining chondrocyte phenotype.

MATERIALS AND METHODS: The lower rim of the scapular (fibrocartilage) of 12-month-old adult NZW rabbits was cut off, cleaned, cut into pieces, and put into a trypsinizing flask containing 0.35% collagenase, 0.05% hyaluronidase, and 0.2% deoxyribonuclease in RPMI medium. The tissue was digested for 4 hours at 37°C to isolate chondrocytes. The cells were collected and re-suspended in 10% RPMI seeded onto a flask. Upon confluency (7-10 days), the cells were trypsinized and further cultured as the followings:

Continued monolayer culture: Cells were re-seeded to a 12-well culture plate with a final cell density of ~3x10^5 cells/ml.

Agarose gel culture: Cells with same final concentration were mixed with 2.5% agarose and let gelled into a 2.5 mm thick film. 12-mm diameter discs were punched out of the film and subsequently cultured in a culture plate.

Polymer gel culture: Cells with same final concentration were seeded in 5% polymer-RPMI media. The media was gelled at 37°C on membrane inserts designed for 12-well culture plates, with a gel thickness of 2.5 mm. The inserts were placed in 12-well plates and cultured for 4 days.

At days 14, the monolayer culture and the agarose discs were stained with Alcian blue, Safranin O, and alkaline phosphatase (ALP) stains to show the presence of proteoglycan in the culture and ALP in the cytoplasm. For the polymer culture, the cells were collected by cooling down the culture and cytospined onto microslides and stained with HE and ALP stains and examined under a light microscope. Also, the number of cells were counted with a hemocytometer.

RESULTS & CONCLUSION: By the day 14, the cells in monolayer culture appeared to be more fibroblast-like. The polymer and agarose gel cultures reexpressed the chondrocyte phenotype. Large amounts of proteoglycan around large oval or round cells was shown in the agarose gel by Alcian blue and Safranin O staining. The positive ALP staining showed the existence of ALP in the cell. On the cytospined slides from the cells recovered from the polymer culture, large oval or round cells were shown by H & E staining and positive ALP staining. The number of cells doubled by 14 days in the polymer culture, from 3x10^5 to 6.6x10^6 cells/ml, indicating the polymer culture not only made the cells regain their chondrocyte phenotype but also promoted cell duplication. It is important to keep the culture in a gelled condition to maintain the association between cells.

In conclusion, the chondrocytes isolated from rabbit scapula can duplicate and reexpress their phenotype in agarose culture and polymer gel culture but not in the monolayer culture. Because the cultured chondrocytes can be easily recovered from the polymer gel by lowering the culture temperature, this new in vitro method for propagation of chondrocytes (patient in processing) is recommended for chondrocytes culture before cell seeding or transplantation.


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PROPAGATION OF CHONDROCYTES USING A THERMOSENSITIVE POLYMER GEL CULTURE

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