Short Communication

Access to Chondrocyte Culture, with Alginate, In Iran

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Abstract

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In this study, chondrocyte culture was established for the first time in Iran, and calcium alginate was used for longer culture of chondrocyte in vitro. The study was programmed in order to be used for future human chondrocyte transplantation. The cartilage specimen obtained from 50 patients who underwent total knee and hip operations in Isfahan University of Medical Sciences. Cartilage specimens were used for monolayer as well as suspension culture in alginate beads. Approximately 12±1 millions cells were harvested from the 3rd passage. The cells were round with large euchromatic nucleus and several nucleoli and small vacuoles. The cells derived from passages 1 to 4, which were grown up then, in alginate beads, showed higher staining with alcian blue. The harvested cells in some patients were immediately and successfully used for autologus transplantation. This later work will be reported separately.

Keywords: Autologus Chondrocyte Transplantation, Cell Culture, Alginate, Proteoglycan

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Due to the lack of arterial blood supply, and venous and lymphatic drainage to the articular cartilage, this tissue takes its nutrition primarily from the synovial fluid and to some extent from the adjacent bone blood supply. So, non-mitotic cartilage and lack of blood supply could be the main causes of inability of this tissue to be self-reparative.

This low potential of self-repair has led to the development of several techniques including autologous chondrocyte transplantation (ACT), which is a modern treatment for articular cartilage defects. Over 12,000 patients have been treated internationally using ACT (1). With ACT, good to excellent results have been reported in post-traumatic isolated lesions of the knee joint (2).

For ACT, a biopsy from the non-bearing area of the joint cartilage or from the area around the damage cartilage is removed and delivered to the ATC laboratory for chondrocyte proliferation. In countries where ACT services are not available, the biopsy specimens are transported in a tube of media to another country where they are proliferated and sent back to be transplanted. This technique, however, was established for the first time in Iran, for immediate transplantation on the patients to repair their knee cartilage defects.

The cartilage specimen were obtained from 50 patients who underwent total knee and hip operations in educational hospitals of Isfahan University of Medical Sciences (IUMS). The patients were between 25 to 70 years old. Following surgery, the specimens were immediately placed in a tube containing PBS and transferred to the ATC laboratory. The specimen were cut into small slices and incubated in Ham`s- F12 medium. After rinsing, cartilage slices were treated first with 1% pronase for two hours at 37ºC and subsequently with 0.2% (v/v) collagenase for 4 hours at 37ºC. Following cell digestion, cells

were centrifuged and washed with culture medium. At first, the cells were counted and briefly investigated for determining the number of live cells with using a drop of Trypan blue on the cells stretched on the hemocytometer slide. Afterward the cells were seeded at 500.000 to 1.000.000 cells per ml, in 60 ml culture flaks at 37ºC and 5% CO2. Media renewal was carried out every 3 days. Upon confluency, cells were passaged using 0.0 5% trypsin/1mM EDTA and re-seeded in flask in 1:3 dilutions. The status of cells was assessed from passage 1 to 8 with Alcian blue staining in order to determine the extent of glycosaminoglycans.

Cells from the each passage were also used for suspension culture in alginate beads. The chondrocytes were suspended in 1.2% sodium alginate solution, at a density of 2 $\times 10^6$ cells per ml. Calcium alginate beads were formed 10 minutes after dripping the cell suspension from a needle in 1.2 mM calcium chloride that each bead contained 400.000 cells (3, 4). The newly formed beads were removed from the calcium chloride solution, rinsed in normal saline solution, and placed on to six well cell culture plates and covered with culture medium. Alginate bead were also cultured at 37ºC and 5% CO2, in a humidified atmosphere. Cells in the alginate beads were also assessed by Alcian blue staining on days 1, 4, 6, 8, 11, 15, and 17.

The morphology of cells obtained from the specimens showed a heterogeneous population. But the cells obtained from younger patients were large and round with a large euchromatic nucleus, several nucleoli and small vacuoles (Fig 1).

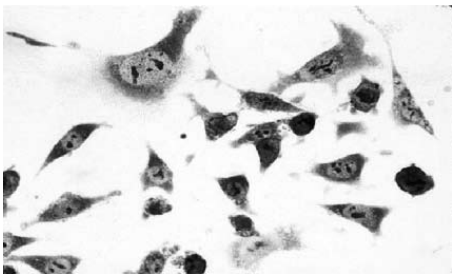

Fig 1: Shows passage 1 of chondrocytes stained with methylene blue (×320) (This Figures has also been printed in full-color at the end issue)

Hematocytometry was used to determine number of harvested cells and to determine percentage trypan blue positive cells. Approximately, 95% of the harvested chondrocytes were alive. In 3rd passage, the cultured chondrocytes reached up to 12±1 millions cells. In some patients isolated chonrocytes were immediately and successfully used for transplantation which will be reported separately.

Observation of alginate bead revealed that the cells began to emerge from alginate and seeded on the culture dish around day 5. The immigrated cells from beads gradually increased and cells covering the dish gradually reached confluence. In this study, the alginate beads derived from passages from 1 to 8 and cells derived from the alginate were stained with Alcian blue, the results of which revealed an increase in staining until passage 4 and then, a decrease (Fig 2).

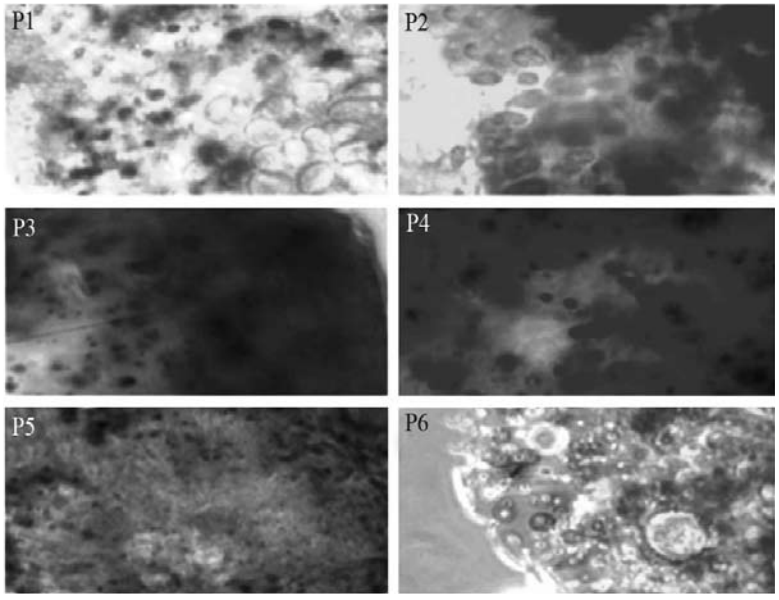

Fig 2: Shows Alcian blue staining for Proteoglycan (PG), increasing production of PG (Passages P1- P4) and decreasing of production, in later passages (Passages P5 -P6) (×400) (This Figures has also been printed in full-color at the end issue)

The results of monolayer chondrocyte culture and chondrocyte alginate bead showed that the production of proteoglycans, from passage 1 to 4 was gradually increased, while from passage 5 to 8, the amount of these substances were tapering down. Therefore, indicating that the best passage for autologus chondrocyte transplanation is passage 2 to 4. Our results are consistent with those of Schultze- Tanzil et al (5) suggesting that the cells undergo dedifferentiation upon further passages.

Various authors showed that chondrocytes in alginate or agarose synthesize a mechanically functional matrix (6) similar to native articular cartilage (7, 8). In summary, the established chondrocyte culture system and chondrocytes alginate system, can provide adequate number of cells for transplantation.

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References

1. Marlovits S, Zeller P, Singer P, Resinger C, Vecsei V. Cartilage repair: generations of autologous chondrocyte transplantation. Eur J Radiol 2006; 57(1): 24-31

2. Robinson D, Ash H, Aviezer D, Agar G, Halperin N, Nevo Z. Autologous chondrocyte transplantation for reconstruction of isolated joint defects: the Assaf Harofeh experience. Isr Med Assoc J 2000; 2(4): 290-295

3. Guo JF, Jourdian GW, MacCallum DK.Culture and growth characteristics of chondrocytes encapsulated in alginate beads. Connect Tissue Res. 1989; 19(2-4): 277-297

4. Hauselmann HJ, Fernandes RJ, Mok SS, Schmid TM, Block JA, Aydelotte MB, et al. Phenotypic stability of bovine articular chondrocytes after long-term culture in alginate beads.J Cell Sci. 1994; 107 (Pt 1): 17-27

5. Schulze-Tanzil G, de Souza P, Villegas Castrejon H, John T, Merker HJ, Scheid A, et al. Redifferentiation of dedifferentiated human chondrocytes in high-density cultures, Cell & Tissue Reseach, 2002; 308(3): 371-379

6. Buschmann MD, Gluzband YA, Grodzinsky AJ, Kimura JH,Hunziker EB. Chondrocytes in agarose culture synthesize a mechanically functional extracellular matrix. J Orthop Res. 1992; 10(6): 745-758

7. Hauselmann HJ, Aydelotte MB, Schumacher BL, Kuettner KE, Gitelis SH, Thonar EJ.Synthesis and turnover of proteoglycans by human and bovine adult articular chondrocytes cultured in alginate beads. Matrix. 1992; 12(2): 116-129

8. Hauselmann HJ, Masuda K, Hunziker EB, Neidhart M, Mok SS, Michel BA,Thonar EJ. Adult human chondrocytes cultured in alginate form a matrix similar to native human articular cartilage. Am J Physiol. 1996; 271(3 Pt 1): C742-752