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Formation of Cartilage Tissue In Vitro

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Abstract Articular cartilage is notoriously defective in its capacity for self-repair, making joints particularly sensitive to degenerative processes. However, methods are now available for the preparation of large numbers of differentiated chondrocytes from a small biopsy sample from any patient. The cells are amplified by proliferation as fibroblast-like cells that will re-express the cartilage phenotype when placed in suspension or gel culture. The chondrocytes can be collected from gel cultures after agarase treatment and reconstituted into cartilage tissue in pellet cultures. In addition, these chondrocytes can be suspended in an appropriate delivery vehicle and implanted into defect sites with a high reparative success rate in an animal model. Appropriate procedures can now be tested in appropriate patient populations.

Key words: chondrogenesis, chondrocyte, cell culture, joint repair

Unlike other musculoskeletal tissues, cartilage lacks the ability to replace damaged tissue with normal cells and extracellular matrix that restore normal function [1]. Even when repair does occur, degeneration often follows [2]. Some experimental approaches indicate that the introduction of stem cells or cartilage cells to the injured site has the potential of improving cartilage repair.

CELL CULTURES

In recent years, a great deal has been learned about the environmental conditions that favor chondrocyte growth and differentiation in tissue culture. This knowledge can now be utilized to engineer cartilage tissue and can be applied to improving cartilage repair.

Two conditions that favor chondrogenesis are high cell density or reduced cell-substratum interactions [3]. Under such conditions, embryonic mesenchymal cells will become chondrocytes and maintain their differentiated properties. For already differentiated chondrocytes, the required conditions are met by suspension cultures [4], culture in gels such as agarose [5–7], or as pellets [8,9].

Depending on the anatomical location, cartilage can persist as permanent cartilage, as in articular cartilage, or undergo maturation to form hypertrophic cartilage, as in the growth plate. These culture conditions considered above promote the maturation of cartilage through the various stages of chondrogenesis [10] to the hypertrophic stage. Maturation of even normally permanent cartilage is observed in gel cultures [11].

However, the recent observation that the formation of hypertrophic cartilage is dependent on a component of the fetal calf serum [12] suggests that it may be possible to maintain immature chondrocytes by utilizing a serum-free culture medium.

Whatever the basis for the environmental enhancement of chondrogenesis by culture under the conditions considered above, the response mechanism might represent a basic cell biological process that could play an essential role in normal chondrogenesis [13]. Benya and Shaffer [14] found that rabbit chondrocytes could be grown extensively as dedifferentiated chondrocytes in order to expand cell numbers and then caused to re-express the chondrocyte phenotype by subculture in agarose. More recently, Aulthouse et al. [15] applied this procedure to human chondrocytes. Small numbers of cells obtained from biopsy specimens could be amplified in monolayer culture and then reverted to chondrocytes in agarose. Such a culture produced in our laboratory is illustrated in Figure 1. The surviving cells proliferate, become immunoreactive for cartilage matrix molecules such as type II collagen, and often become encased in a proteoglycan-rich extracellular matrix.

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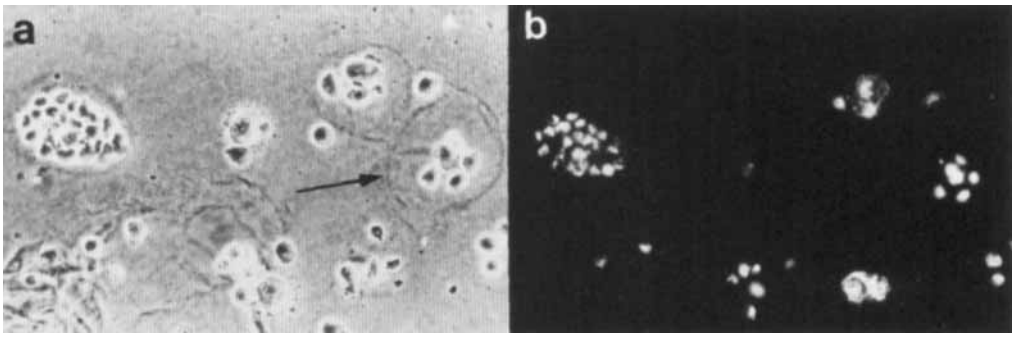

Fig. 1. Agarose gel culture of human chondrocytes. **a:** Phase contrast micrograph. **b:** Immunofluorescence micrograph for type II collagen. Initially, fibroblast-like cells were derived from an explant culture of a normal tibial growth plate from a 4 year old patient, according to the procedure described by Aulthouse et al. [15]. The cells were stored frozen after the third subculture, thawed 2 ½ years later, and grown in monolayer culture for 5 days. Then the cells were trypsinized and placed in 0.5% low-temperature agarose culture according to Benya and Shaffer [14], in DMEM containing 10% fetal calf serum and antibiotics. After 21 days, the cultures were fixed and stained for type II collagen with a mouse monoclonal antibody kindly provided by Dr. T.F. Linsennayer, as described previously [11]. Note that most of the cells are immunoreactive and some clusters are surrounded by a sphere of extracellular matrix (arrow). The cells that are negative for type II collagen are probably dead since cells immunoreactivity for type I collagen was not observed in the gel culture. 130×.

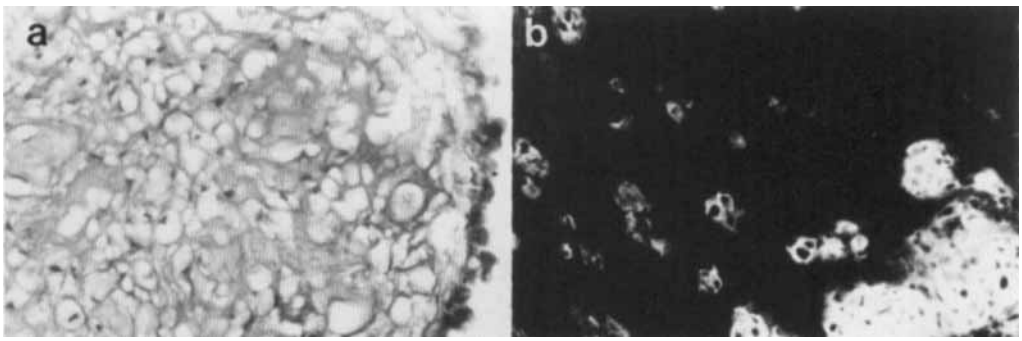

Fig. 2. Sections of human chondrocyte pellet culture: (**a**) Alcian blue (pH 1) stained, (**b**) immunofluorescence micrograph for type II collagen. Sister cultures to those shown in Figure 1 were treated with dilute agarase in culture medium overnight at 37°C on day 12 of agarose culture. The cells were dispersed into culture medium, pelleted by centrifugation in a 15 ml tube, and cultured in 2 ml of medium as a pellet, as described by Kato et al. [9]. The medium, containing 50 μg/ml ascorbate, was changed every 2 days for 30 days, when the cultures were fixed in Carnoy's fixative and paraffin sectioned. a, 215×; b, 130×.

We have found that chondrocytes can be readily recovered from agarose cultures by the use of agarase and subsequently placed in pellet cultures according to the method of Kato et al. [9]. Under these conditions, a piece of pure cartilage tissue can be reconstituted (Fig. 2). Such cultures can be used for molecular, biochemical, metabolic, or mechanical studies of normal or abnormal cartilage.

In the present context, the ability to recover large numbers of differentiated chondrocytes from a small biopsy sample makes it feasible to carry out autologous cartilage grafting. Large numbers of fibroblast-like cells can be grown from small biopsies of growth-plate or articular cartilage, and the derived cells can be frozen until needed. The thawed cells will subsequently re-express the cartilage phenotype in agarose gels and can be collected as a cell suspension or as an appropriately shaped piece of cartilage.

IMPLANTS

Development of appropriate implant procedures is an area that needs further exploration. There have been numerous studies over the years along these lines with only limited success in the past [16]. Cartilage fragments are difficult to implant and often degenerate. Mesenchymal cells often give rise to cartilage as well as to fibrous tissues, resulting in poor integration.

Recently, Robinson et al. [17] have had success in an avian model by obtaining differentiated chondrocytes from suspension culture. The best results were obtained by suspending the cells in a concentrated solution of hyaluronic acid, which serves to protect the cells in a biocompatible, permissive matrix that can be readily implanted. In these studies, long-term repair of full thickness defects were obtained with a 75% success rate. Improved success could probably be obtained by the use of continuous passive movement of the joint [18] and perhaps by the inclusion of chondro-osteoinductive factors, including members of the TGF-β family [19–22], in the implant material.

CONCLUSIONS

As a result of basic cell biological studies on chondrogenesis, cell culture methods are now available for the production of large numbers of cartilage cells from any individual. This technological capability is ripe for application to cellular therapies for a variety of joint diseases including osteoarthritis. Human trials should be undertaken.

Still, a number of important variables need careful exploration. These include determination of the best cell source for the reliable and efficient recovery of chondrocytes, the development of improved implant vehicles and additives to ensure optimal integration, and long-term repair and a better understanding of the factors that regulate chondrocyte maturation.

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REFERENCES

1. Buckwalter J, Rosenberg L, Coutts R, Hunziker E, Reddi AH, Mow V: In Woo S.L.-Y., Buckwalter J (eds): "Injury and Repair of the Musculoskeletal Soft Tissues." Park Ridge, IL: American Academy of Orthopaedic Surgeons, 1987, pp 465–482.
2. Furukawa T, Eyre DR, Koide S, Glimcher MJ: J Bone Joint Surg 62A: 79–89, 1980.
3. Zanetti NC, Solursh M: In "Cell Shape: Determinants, Regulation, and Regulatory Role." New York: Academic Press, 1989, pp 291–327.
4. Horwitz AL, Dorfman A: J Cell Biol 45:434–438, 1970.
5. Aydelotte MB, Keuttner KE: Connect Tissue Res 18: 205–222, 1988.
6. Delbruck A, Dresow B, Gurr E, et al.: Connect Tissue Res 15:115–172, 1986.
7. Watt FM, Dudhia J: Differentiation 38:140–147, 1988.
8. Manning WK, Bonner WM Jr: Arthritis Rheum 10:235–239, 1967.
9. Kato Y, Iwamoto M, Koike T, Suzuki F, Takano Y: Proc Natl Acad Sci USA 85:9552–9556, 1988.
10. Franzen A, Heinegard D, Solursh M: Differentiation 36:199–210, 1988.
11. Solursh M, Jensen KL, Reiter RS, Schmid TM, Linsenmayer TF: Dev Biol 117:90–101, 1986.
12. Bruckner P, Hörler I, Mendler M, Houze Y, Winterhalter KH, Eich-Bender SG, Spycher MA: J Cell Biol 109: 2537–2545, 1989.
13. Solursh M: Am J Med Genet 34:30–34, 1989.
14. Benya PD, Shaffer JD: Cell 30:215–224, 1982.
15. Aulthouse AL, Beck M, Griffey E, Sanford J, Arden K, Machado MA, Horton WA: In Vitro Cell Dev Biol 25:659–668, 1989.
16. Nevo Z, Robinson D, Halperin N: In Hall BK (ed): "Fracture Repair and Regeneration." Caldwell, NJ: The Telford Press, in press.
17. Robinson D, Halperin N, Nevo Z: Calcif Tissue Int 46:246–253, 1990.
18. O'Driscoll SW, Salter RB: Clin Orthop 208:131–140, 1986.
19. Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kritz RW, Hewick RM, Wang EA: Science 242:1528–1534, 1988.
20. Sampath TK, Coughlin JE, Whetstone RM, Banach D, Corbett C, Ridge RJ, Ozkaynak E, Oppermann H, Rueger DC: J Biol Chem 265:13198–13205, 1990.
21. Joyce ME, Roberts AB, Sporn MB, Bolander ME: J Cell Biol 110:2195–2207, 1990.
22. Vukicevic S, Luyten FP, Reddi AH: Proc Natl Acad Sci USA 86:8793–8797, 1989.