Journal of Cellular Biochemistry 51:257–264 (1993)

Cultivation of Cell-Polymer Cartilage Implants in Bioreactors

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Abstract Cartilage implants for potential use in reconstructive or orthopedic surgery can be created by growing isolated cartilage cells (chondrocytes) in vitro on synthetic, biodegradable polymer scaffolds. The scaffolds provide specific three-dimensional structures which support cell proliferation and biodegrade in a controlled fashion in parallel to cellular regeneration of cartilaginous tissue. Cartilage implants based on chondrocytes and fibrous polyglycolic acid scaffolds were recently shown to closely resemble normal cartilage histologically as well as with respect to cell density and matrix composition (collagen, glycosaminoglycan) [Freed et al., J Biomed Mater Res 27:11–23, 1993a]. These findings form the basis for developing straightforward procedures to obtain implants for clinical use from small, autologous cartilage specimens without any limitations in terms of availability of donor tissue or implant dimensions.

Chondrocyte growth and cartilage matrix regeneration on polymer scaffolds are interdependent and also depend on in vitro tissue culture conditions. Under static culture conditions, cell growth rates are diffusionally limited due to increasing cell mass and decreasing effective implant porosity resulting from cartilage matrix regeneration. Optimization of the in vitro culture environment is thus essential for the cultivation of large, clinically useful cartilage implants. Preliminary studies indicate that major improvements can be achieved using bioreactors that provide efficient mass transfer and controlled shear rates at the cell and implant surfaces.

Key words: bioreactor, chondrocyte, chondrogenesis, tissue engineering, cartilage, joint repair

Reconstructive plastic and orthopedic surgeries are done on a daily basis at major medical centers in order to treat the many patients with damaged cartilage due to congenital abnormalities or trauma [Bently, 1989; Zukor et al., 1990]. Current therapies include 1) transplantation (i.e., excising healthy host cartilage, carving it into the desired shape and reimplanting it where needed) and 2) implantation of artificial prosthetic devices. However, the amount of donor tissue available for transplantation is limited, and it is often not possible to form delicate three-dimensional (3-D) implants from the harvested tissue. Artificial prostheses are complicated by infections and a high incidence of adhesive breakdown at the host/prosthesis interface. Also, a prosthesis cannot adapt in response to environmental stresses as does living cartilage tissue. The clinical need for improved treatment options in reconstructive surgery has motivated research aimed at the in vitro cultivation of isolated chondrocytes on polymer scaffolds to regenerate cartilage for clinical implantation. The ability to use autografts eliminates the need for potentially dangerous immunosuppressive drugs in the envisioned clinical application.

Cartilage regeneration has been reported in vivo using systems based on isolated chondrocytes [Chesterman and Smith, 1968; Grande et al., 1989; Takigawa et al., 1987], organic support matrices [Nevo et al., 1992; Wakitani et al., 1989], and synthetic polymer scaffolds [Freed et al., 1993; Vacanti et al., 1991]. Subcutaneous injection of isolated chondrocytes resulted in small cartilaginous nodules [Freed et al., 1993; Takigawa et al., 1987]. Entrapment of isolated chondrocytes within a focal cartilage defect (using a perichondrial flap) resulted in cartilage regeneration, but this method was technically difficult and the dimensions of the repair tissue were limited [Grande et al., 1989; Brittberg et al., 1992]. In order to resurface a larger cartilage defect, a scaffold can be used to provide a 3-D structure for in vitro chondrocyte proliferation and to control the shape of a regenerated carti-

Received September 2, 1992; accepted September 10, 1992.

lage "implant" for in vivo use. A damaged joint surface could potentially be repaired with such an implant, if its form and function resembled those of normal cartilage (i.e., it contained sulfated glycosaminoglycans (GAG) and type II collagen, and had appropriate biomechanical strength).

In our laboratory, cartilage implants have been created using chondrocytes cultured on synthetic, biodegradable, 3-D polymeric scaffolds (Fig. 1) [Freed et al., 1993]. Fibrous polyglycolic acid (PGA) proved to be an excellent scaffold for tissue engineering. Within PGA scaffolds, bovine and human chondrocytes proliferated and regenerated cartilaginous "implants" both in vitro (in Petri dishes) and in vivo (subcutaneously in nude mice). 6 weeks in vitro implants reached a cellularity equal to that of the parent cartilage specimen, contained GAG and collagen, and had the histological appearance of normal cartilage. 1–6 months in vivo implants appeared glistening white macroscopically, and consisted only of cells and cartilaginous matrix [Freed et al., 1993].

Ideally, a chondrocyte-polymer system for cartilage regeneration should be based on 1) a small quantity of donor tissue, 2) a cell-compatible scaffold with a clinically useful structure, and 3) a bioreactor in which the cells can efficiently proliferate and regenerate cartilage matrix in vitro, where a bioreactor is defined as a tissue culture vessel in which the contents are well mixed, and the culture conditions can be controlled over the entire cultivation period. Specifically, for (1)–(3) above:

1) Cells obtained from small needle-biopsy specimens ($\sim$50 mg) of donor tissue must be amplified in order to generate large autograft implants for clinical use. This is because chondrocytes must be cultured at high cell densities to maintain their differentiated phenotype [Bruckner et al., 1989; Solursh 1991; Tachetti et al., 1992]. Cells can be amplified either by serial passage using petri dishes, or in a single pass using bioreactors. However, the former is labor intensive and causes chondrocytes to dedifferentiate [Bruckner et al., 1989; Solursh, 1991], while bioreactors can support higher cell growth rates due to more efficient mass transfer of nutrients and wastes [Bugarski et al., 1992; Posillico, 1986; Thilly et al., 1982].

2) A biocompatible scaffold is required to provide a 3-D structure for the regeneration of a cartilage implant from isolated chondrocytes [Freed et al., 1993; Nevo et al., 1992]. The presence of the scaffold is only required for the first 2–3 weeks of cell culture during which time enough extracellular matrix is formed to give the implant mechanical integrity. After this, the scaffold should biodegrade, to permit the regeneration of a compact tissue, and to minimize the host inflammatory response upon in vivo implantation. Highly porous scaffolds are required to minimize diffusional constraints during chondrocyte growth, and to provide sufficient space for cartilage matrix regeneration; 3-D scaffolds with clinically relevant dimensions and structures are more reproducibly formed using synthetic (e.g., PGA) instead of naturally occurring biomaterials (e.g., agarose).

3) An in vitro tissue culture environment is required which can support the growth of clinically useful cartilage implants. These can be quite large, for example, the cartilage surface of the femur in the human knee can be considered as a 5 cm diameter cartilage disc with variable thickness (0.1–0.5 cm) and a volume of about 5 cm^3. The four processes which are occurring simultaneously during the in vitro cultivation of such an implant are 1) chondrocyte proliferation, 2) cartilage matrix regeneration (GAG, collagen), 3) scaffold degradation, and 4) decreasing porosity and increasing mass transfer limitations due to processes (1) and (2) above.

IN VITRO CARTILAGE REGENERATION

Effects of Cell and Scaffold Parameters

A chondrocyte-polymer system for the regeneration of cartilage implants for in vivo use depends on 1) cell density, 2) scaffold degradation rate, 3) scaffold porosity, and 4) scaffold thickness [Freed et al., 1993 in preparation].

High cell densities were required to regenerate compact, functional cartilage tissue [Freed et al., 1993a]. This finding is consistent with previous reports that chondrocytes cultured at high densities express more differentiated phenotypes [Bruckner et al., 1989; Solursh, 1991; Tachetti et al., 1992] and can be attributed to cell-to-cell and cell-to-extracellular matrix interactions known as the "community effect" [Gurdon, 1988]. High implant GAG contents were associated with high chondrocyte densities and vice versa (Table I). Implant GAG contents were also related to implant biomechanical properties: the higher the GAG content, the less the implant was deformed when subjected to constant compressive stress [Freed, in prepara-

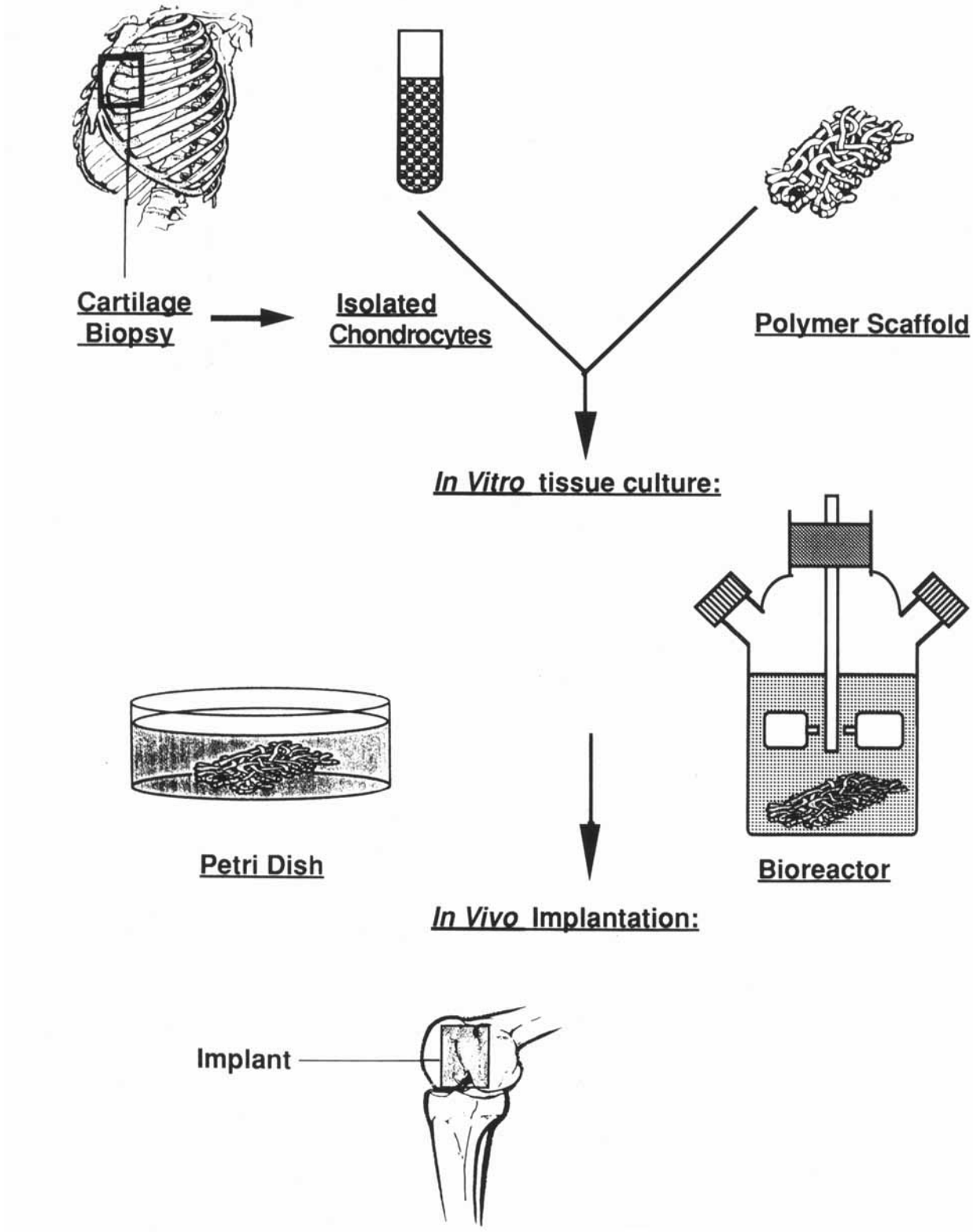

Fig. 1. *Proposed therapy:* A cartilage sample is enzymatically digested to yield component chondrocytes which are cultured in vitro on synthetic, biodegradable scaffolds in Petri dishes or bioreactors, and then implanted in vivo where needed.

TABLE I. Chondrocyte-Polymer Implants in Petri Dishes

	Scaffold dimensions		Implant properties		Chondrocyte growth kinetics		
	Thickness (cm) initial	Volume (cm^3) initial	Cell density (cells/cm^3 implant) 23 days	GAG content (mg/cm^3 implant) 23 days	Cell growth rate[a] (cells/cm^3/day) 2 days	23 days	Doubling time[b] (days) 2 days
Group							
1	0.088 ± 0.005	0.069	14 ± 0.56 × 10^7	23.2 ± 0	8.69 × 10^6	2.71 × 10^6	1.913
2	0.116 ± 0.005	0.091	10 ± 0.52 × 10^7	11.1 ± 0.85	6.39 × 10^6	1.16 × 10^6	2.275
3	0.168 ± 0.009	0.132	6.6 ± 0.22 × 10^7	7.64 ± 0.33	3.92 × 10^6	1.06 × 10^6	2.609
4	0.307 ± 0.004	0.241	4.1 ± 0.29 × 10^7	6.02 ± 0.26	2.43 × 10^6	0.24 × 10^6	5.286
5	0.384 ± 0.013	0.302	3.5 ± 0.21 × 10^7	4.94 ± 0.27	2.29 × 10^6	0	5.721

[a]Cell growth rates, R(t), were calculated from 2nd order polynomial fits of measured chondrocyte concentration profiles, X(t), according to $R(t) = R_o - kt$, where R_o equals initial cell growth rate (cells per cm^3 implant per day), and k equals the rate at which the cell growth rate decreased with cultivation time.
[b]Doubling time, t_d, was calculated according to $t_d = X(t)/R(t)$.

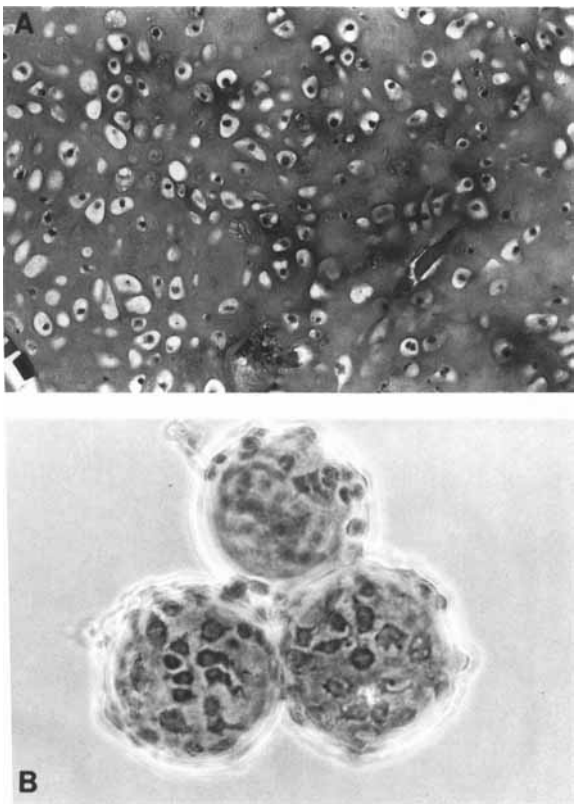

Fig. 2. **A:** Chondrocyte-polymer cartilage implant (5 μm thick histological section stained with safranin-O, ×100). **B:** Chondrocytes growing on microcarrier beads (stained with crystal violet, ×200).

tion]. This finding is consistent with studies of normal cartilage explants and chondrocytes cultured in agarose gels [Mow et al., 1992; Buschmann et al., 1992].

Scaffolds which had higher porosities and faster degradation rates were better for cartilage regeneration than those with low porosities and slow degradation rates. Specifically, chondrocytes proliferated and produced GAG more rapidly within PGA meshes (98% porous, 80% degradation of mass over 2 months) than within polylactic acid (PLA) sponges (91% porous, no degradation over 2 months) [Freed et al., 1993a]. Thin (<0.17 cm) chondrocyte-PGA implants grown in vitro for 30 days consisted of uniformly distributed cells surrounded by a cartilaginous matrix; only microscopic remnants of the PGA scaffold were observed (Fig. 2A). The extracellular matrix stained positive for glycosaminoglycan (GAG) and types I and II collagens. In contrast, thick (>0.3 cm) implants also grown in vitro for 30 days contained large quantities of undegraded PGA surrounded by a tissue-like periphery. Chondrocyte and GAG densities were also significantly higher for thin than for thick scaffolds (Table I).

Under static culture conditions, chondrocyte proliferation within polymer scaffolds followed the characteristic pattern observed for diffusionally controlled processes [Chang and Moo-Young, 1988; Weisz, 1973]. Experimentally determined cell growth rates decreased with both increasing scaffold thickness and increasing cultivation time (Fig. 3). Doubling times increased significantly with increased implant thickness (Fig. 4). This resulted in four times as many cells per unit implant volume in thin (0.088 cm) as in thick (0.384 cm) 23 d implants (Table I, group 1 vs. 5). These findings are consistent with previous reports that high density cell cultures under static culture conditions (Petri dishes) are often limited by inadequate supply of nutrients and oxy-

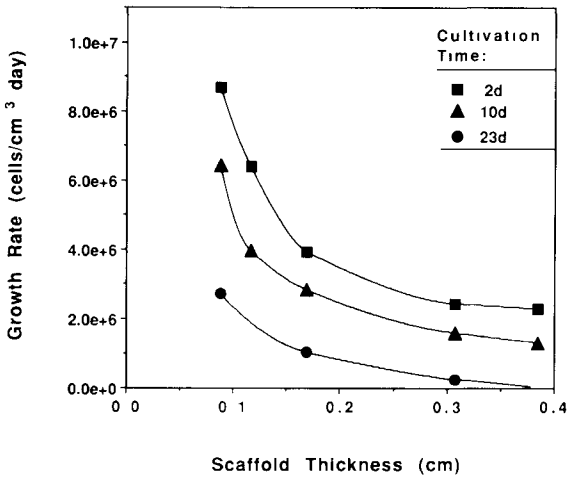

Fig. 3. Effects of scaffold thickness and implant cultivation time on cell growth rates.

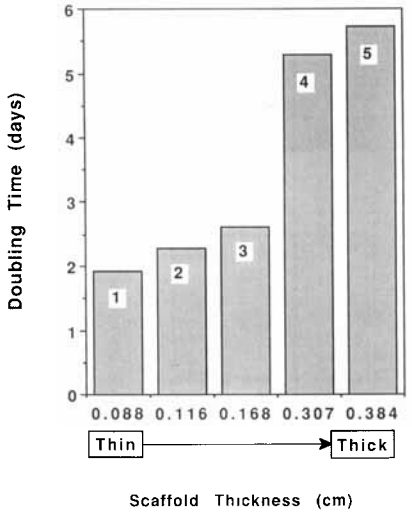

Fig. 4. Effect of scaffold thickness on cell doubling time.

gen [Bugarski et al., 1993; Posillico, 1986; Thilly et al., 1982].

Effects of In Vitro Culture Conditions

Under well-mixed culture conditions, mass transfer rates can be maintained at higher levels and cell growth is not restricted by the rate of nutrient supply, as compared to static culture conditions. The importance of in vitro culture conditions was demonstrated using a model system: chondrocytes grown on microcarriers in bioreactors (Fig. 2B). Specifically, 5×10^5 cells/cc were inoculated into bioreactors containing 5 mg/cc of microcarriers (Cytodex III collagen coated spheres, 175 μm diameter). Bioreactors which were mixed by magnetic stirring or by shaking were compared to unmixed test tubes (Table II). The intensity of stirring or shaking was maintained at the minimum level required for spatially uniform suspension of the microcarriers. Growth constraints (nutrients and waste toxicity) were minimized by daily medium renewal (50% per day). Under these conditions, cells remained viable on microcarriers for 4 months to date. In contrast, cultures which were not refed were not viable after 2 weeks.

In bioreactors (Table II, groups A and B), the microcarriers remained uniformly suspended for the first 8 days of cultivation, after which they formed macroscopic aggregates consisting of beads interconnected by cells and extracellular matrix. Initial cell attachment to microcarriers depended on the mixing method: cell densities at days 2 and 8 in the magnetically stirred flask were ten times those in the shaking flask (Table II, groups A and B, respectively). As shown in Figure 5, initial cell doubling rates were similar (1–2 days) in both bioreactors (groups A and B), and much slower in the unmixed test tubes (4.9 days at day 2 increasing to infinite by day 8) (Table II, group C). The overall increase in cell mass between days 2 and 8 was 10- to 12-fold in bioreactors, but only by 1.5-fold in unmixed test tubes.

In both static Petri dishes and well-mixed bioreactors, lower chondrocyte densities were associated with shorter cell doubling times and

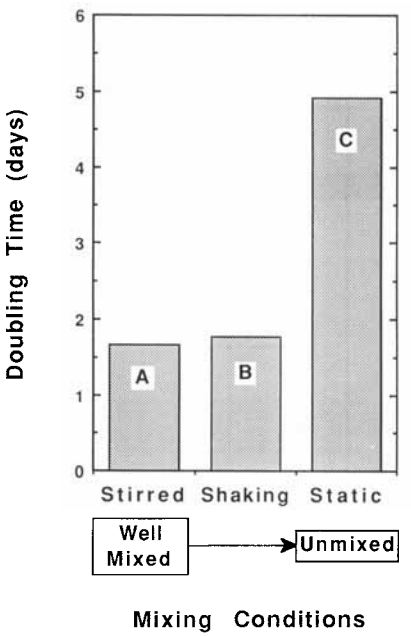

Fig. 5. Effect of mixing conditions on cell doubling time.

TABLE II. Chondrocyte Growth on Microcarriers in Bioreactors

Group	Bioreactor	Cell density (cells/cm³ reactor volume) 2 days	Cell density (cells/cm³ reactor volume) 8 days	Doubling time[a] (days) 2 days	Doubling time[a] (days) 8 days
A	Magnetically stirred flask, (75 rpm)	1.30×10^5	1.58×10^6	1.67	1.67
B	Shaking flask (140 rpm)	1.49×10^4	1.54×10^5	1.78	1.78
C	Unmixed test tubes	1.98×10^5	2.96×10^5	4.91	∞

[a]In groups A and B, doubling times, t_d, were calculated from exponential fits of measured cell concentrations according to $t_d = (\log 2)/k$, where k equals the kinetic rate constant. In group C, doubling times were calculated from 2nd order polynomial fits of measured cell concentrations, as described in Table I.

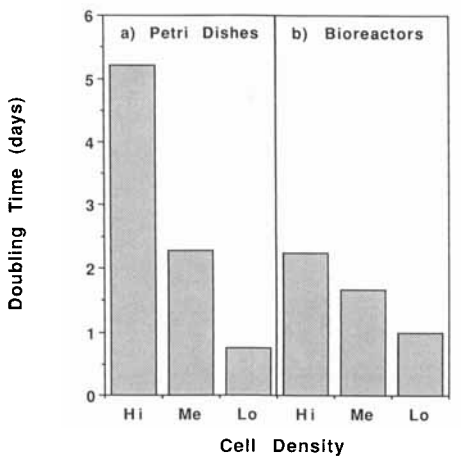

Fig. 6. Effects of cell density on cell doubling time: a) in Petri dishes and b) in bioreactors.

TABLE III. Effects of Cell Density and Culture Conditions on Chondrocyte Growth

Culture conditions	Cell density	Doubling time
Petri dishes	(cells/cm³ implant) 2 days	(days) 2 days
High	$2.92 \pm 0.18 \times 10^7$	5.205
Medium	$1.45 \pm 0.08 \times 10^7$	2.275
Low	$0.40 \pm 0.14 \times 10^7$	0.751
Bioreactors	(cells/cm³ reactor volume) 2 days	(days) 2 days
High	2.65×10^5	2.24
Medium	1.30×10^5	1.67
Low	1.74×10^4	1.00

vice versa (Fig. 6, Table III). It is likely that the cells proliferate faster at lower cell densities in order to establish a critical cell mass prior to expressing a differentiated phenotype [Bruckner et al., 1989; Zanetti and Solursh, 1989]. This effect was much more pronounced under static culture conditions due to even less sufficient nutrient mass transfer on a per cell basis. Cell growth kinetics were qualitatively similar under static and mixed culture conditions; only implants with low cell densities ($< 5 \times 10^6$ cells/cm³) and at the beginning of their in vitro cultivation were not diffusionally limited (Table III). Creation of uniform implants with high cell densities thus requires bioreactors which can overcome considerable mass transfer limitations.

CARTILAGE REGENERATION IN BIOREACTORS

Bioreactors have been developed in various designs and capacities for various biotechnological applications. For example, large bioreactors (up to 10^5 L) are routinely used for microbial cell production of biologicals (e.g., penicillin). Small bioreactors (≤ 0.5 L) for tissue engineering are currently at an early developmental stage. The use of bioreactors for tissue engineering could offer several potential advantages as compared to static Petri dishes: 1) uniform and efficient mixing coupled with precise control over mass transfer rates; 2) regulation of shear stress within the vessel; 3) maintenance of constant pH, gas partial pressures (pO_2, pCO_2) and nutrient levels (e.g., glucose); and 4) process control strategies which can match the changing needs of a growing implant over the entire duration of its cultivation.

Bioreactors mixed by mechanical stirring are routinely used to culture mammalian cells, as long as the impellers are properly designed and mixing speeds are slow [Thilly and Levine, 1979; Thilly et al., 1982]. However, with mechanical stirring, the mixing intensity varies widely: the shear rate is 10-fold higher at the impeller

surface than elsewhere within the bioreactor [Merchuk, 1990]. These shear gradients are associated with nutrient and pH gradients and non-uniform mass transfer rates which could adversely effect cell growth and function. In addition, the impeller can itself interfere with cell-scaffold interactions in the early stages of implant cultivation. Thus, bioreactors which rely on mechanical mixing may not be optimal for growing tissue-like implants for clinical use.

Novel bioreactor designs which rely on mixing by secondary fluid flow instead of mechanical stirring have recently been proposed. For example, a uniform microenvironment can be achieved in bioreactors mixed by fluid recirculation [airlift reactors (ALR), fluidized bed reactors (FBR)] [Hu, 1992]. When microencapsulated hybridoma cells were cultured in either ALR or FBR designs, both final cell densities and monoclonal antibody production levels were 10-fold higher than when the same cells were cultured in shaking flasks [Bugarski et al., 1993; Poscillico, 1986]. Mass transfer rates were increased over the course of the culture by increasing the rate of internal fluid recirculation.

Rotating microgravity bioreactors (RMB) have recently been developed in which rotation of the inner of two concentric cylinders induces secondary flows in the annularly spaced chamber that mix and suspend the bioreactor contents [Schwarz et al., 1992]. Human colon adenocarcinoma cells grown in a RMB formed 0.2–0.5 cm diameter cell-microcarrier aggregates in which the cells appeared polypoid and contained apical microvilli and glands which secreted mucin [Goodwin et al., 1991]. Differentiated cell function was attributed to the microgravity-like conditions within the bioreactor; the total force per unit of cross-sectional area on an aggregate in microgravity was about one order of magnitude smaller than the corresponding value in unit gravity [Tsao et al., 1992].

In the case of cartilage regeneration, the ability to apply small, controlled shear stresses might also improve the biomechanical properties of the growing implant, since architectural repair of damaged cartilage depends in part on mechanical stresses such as those induced by joint motion [Salter, 1990]. Bioreactor mixing conditions should thus be optimized to maintain the proper balance between mass transfer rates and shear stresses over the course of chondrocyte proliferation and cartilage regeneration. The well-mixed bioreactor designs described above (magnetically stirred flasks, ALR, RMB) are currently being tested with chondrocyte-polymer cartilage implants [Freed et al., in preparation].

SUMMARY AND FUTURE DIRECTIONS

Biotechnology holds promise for both advancing the basic understanding of cell growth and function, and developing new tissue implants to treat human diseases. A method to repair damaged cartilage is proposed in which cells and polymer scaffolds are used to create clinically useful 3-D implants starting from small, autologous "needle" biopsy specimens. Our studies of chondrocyte-polymer implants to date demonstrate the dependence of the size and quality of the final cartilage product on the scaffold and on the tissue culture conditions. Future work should thus focus on two aspects: 1) fundamental studies aimed at relating chondrocyte growth and differentiation to tissue culture environment, and 2) evaluation of the optimal conditions for bioreactor cultivations of clinically useful cartilage implants. Major improvements in the in vitro cultivation of cells and tissues have been demonstrated using bioreactors which are mixed either by internal liquid recirculation or by external rotation.

Cartilage regeneration from autologous chondrocytes and degradable scaffolds could represent a relatively straightforward procedure to make implants with desired characteristics without any limitations in terms of availability of donor tissue or final implant dimensions. A well-mixed bioreactor system for the cultivation of chondrocytes on polymer scaffolds is expected to allow in vitro regeneration of autologous cartilage implants which can meet specific clinical needs. Specifically designed, functional cartilage implants could form the basis of new cartilage repair procedures in reconstructive plastic or orthopedic surgery. The same approaches and methodologies could also be extended to other cell-polymer model systems and should enhance our understanding the principles governing both normal and cancerous tissue growth and development. Research utilizing bioreactors is thus expected to provide new information applicable to basic biological sciences and biomedical engineering.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health (N.I.H. fellowship F32GM14191

to L. Freed) and Neomorphics Inc. (Lexington, MA). The authors thank J.C. Marquis and M. Lyman for their help.

REFERENCES

Bently G (1989) Grafts and implants for cartilage repair and replacement Crit Rev Biocompat 5 245–267

Brittberg M, Peterson L, Lindahl A, Nilsson A, Olsson C, Isaksson O (1992) Treatment of full thickness cartilage defects in the human knee with autologously isolated and cultured chondrocytes 19th Symposium of the European Society for OsteoArthritis book of abstracts #F7

Bruckner P, Horler I, Mendler M, Houze Y, Winterhalter KH, Bender S, Spycher MA (1989) Induction and prevention of chondrocyte hypertrophy in culture J Cell Biol 109 2537–2545

Bugarski B, Jovanovic G, Vunjak-Novakovic G (1992) Bioreactor systems based on microencapsulated animal cell cultures In Goosen MFA (ed) "Fundamentals of Animal Cell Immobilization and Microencapsulation" Boca Raton CRC Press, pp 267–

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

Chang HN, Moo-Young M (1988) Estimation of oxygen penetration depth in immobilization cells Enzyme Microbiol Technol 29 107–112

Chesterman PJ, Smith AU (1968) Homotransplantation of articular cartilage and isolated chondrocytes an experimental study in rabbits J Bone Joint Surg 50B 184–197

Freed LE, Marquis JC, Nohria A, Emmanual J, Mikos AG, R Langer R (1993) Neocartilage formation in vitro and in vivo using cells cultured on synthetic biodegradable polymers J Biomed Mater Res 27 11–23

Freed LE, Emmanual J, Marquis JC, R Langer R (in preparation) Cartilage regeneration on polymer scaffolds

Goodwin TJ, Jessup JM, Wolf DA (1992) Morphological differentiation of colon carcinoma cell lines HT-29 & HT-29KM in rotating-wall vessels In Vitro Cell Dev Biol 28A 47–60

Grande D, Pitman MI, Peterson L, Menche D, Klein M (1989) The repair of experimentally produced defects in rabbit articular cartilage by autologous chondrocyte transplantation J Orthop Res 7 208–218

Gurdon JB (1988) A community effect in animal development Nature 336 772–774

Hu W-S (1992) Bioreactors for animal cell cultivation In Vardar-Sukan F, Sukan SS (eds) "Recent Advances in Biotechnology" Netherlands Kluwer, pp 243–261

Merchuk JC (1990) Why use air-lift bioreactors? Trends Biotechnol 8 66–71

Mow VC, Ratcliffe A, Poole AR (1992) Cartilage and diarthrodial joints as paradigms for hierarchical materials and structures Biomaterials 13 67–97

Nevo Z, Robinson D, Halperin N (1992) The use of grafts composed of cultured cells for repair and regeneration of cartilage and bone In Hall BK (ed) "Bone Fracture Repair and Regeneration" Boca Raton CRC Press, pp 123–152

Posillico EG (1986) Microencapsulation technology for large-scale antibody production Bio/Technology 4 114–117

Salter RB (1990) The biological concept of continuous passive motion of synovial joints The first 18 years of basic research and its clinical application In Ewing JW (ed) "Articular Cartilage and Knee Joint Function" New York Raven Press, pp 335–353

Schwarz RP, Goodwin TJ, Wolf DA (1992) Cell culture for three-dimensional modeling in rotating-wall vessels An application of simulated microgravity J Tissue Culture Methods 14 51–58

Solursh M (1991) Formation of cartilage tissue in vitro J Cell Biochem 45 258–260

Tacchetti C, Tavella S, Dozin B, Quarto R, Robino G, Cancedda R (1992) Cell condensation in chondrogenic differentiation Exp Cell Res 200 26–33

Takigawa M, Shirai E, Fukuo K, Tajima K, Mori Y, Suzuki F (1987) Chondrocytes dedifferentiated by serial monolayer culture form cartilage nodules in nude mice Bone Mineral 2 449–462

Thilly WG, Levine DW (1979) Microcarrier culture a homogeneous environment for studies of cellular biochemistry In Jakoby WB, Pastan I (eds) "Methods in Enzymology" New York Academic, Vol 58, pp 184–194

Thilly WG, Barngover D, Thomas JN (1982) Microcarriers and the problem of high density cell culture In "From Gene to Protein Translation Into Biotechnology" New York Academic, pp 75–103

Tsao YD, Goodwin TJ, Wolf DA, Spaulding GF (1992) Responses of gravity level variations on the NASA/JSC bioreactor system The Physiologist 35 49–50

Vacanti C, Langer R, Schloo B, Vacanti JP (1991) Synthetic biodegradable polymers seeded with chondrocytes provide a template for new cartilage formation in vivo Plast Reconstr Surg 88 753–759

Wakitani S, Kimura T, Hirooka A, Ochi T, Yoneda M, Yasui N, Owaki H, Ono K (1989) Repair of rabbit articular surfaces with allograft chondrocytes embedded in collagen gel J Bone Joint Surg 71B 74–80

Weisz PB (1973) Diffusion and chemical transformation Science 179 433–440

Zanetti N, Solursh M (1989) Effect of cell shape on cartilage differentiation In Stein WD, Bronner F (eds) "Cell Shape Determinants, Regulation and Regulatory Role" San Diego Academic, pp 291–327

Zukor DJ, Oakeshott RD, Brooks PJ, McAuley JP, Langer F, Gross AE (1990) Fresh, small fragment osteochondral allografts for posttraumatic defects of the knee In Ewing JW (ed) "Articular Cartilage and Knee Joint Function Basic Science and Arthroscopy" New York Raven Press, pp 153–165